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MORPHISM AND EVOLUTION

JULIAN HUXLEY *

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1. INTRODUCTION

EARLY in his career, William Bateson showed the preoccupation with discontinuity which lasted the rest of his life. He became convinced that discontinuous was often more important than continuous variation in producing new forms in evolution, and amassed the huge volume of data included in *Materials for the Study of Variation* (1894) in order to prove it.

On page 76 of this work, he pointed out that " so long as systematic experiments in breeding are wanting, and so long as the attention of naturalists is limited to the study of normal forms, in this part of biology [the occurrence of discontinuous variation], which is perhaps of greater theoretical and even practical importance than any other, there can be no progress." He thus, by a natural transition, became interested in the inheritance of discontinuous variations and was, one might almost say, pre-adapted to become the protagonist and apostle of discontinuity in inheritance, after the rediscovery of Mendel's work in 1900.

He further pointed out (p. ix) the danger of discussing " the causes of Variation and the nature of ' Heredity ', a subject of extreme and peculiar difficulty ", without adequate knowledge of the actual " modes of Variation or of the visible facts of Descent." In passing, we may note the curious fact pointed out by Darlington (1953) that, though Bateson was fascinated by the idea of inheritance being discontinuous, he resisted the idea that it was particulate.

Later, in his *Problems of Genetics* (1913) he was busy noting examples of discontinuous genetic variation and inheritance in nature ; and towards the end of his life was attracted to the problems of discontinuity posed by such curiosities of variation as those of chimaeras, rogue peas and root-cuttings.

It is perhaps not unfair to say that he preferred his problems to retain an element of strangeness and inexplicability, tending to lose interest in them when simple principles of explanation became available. However that may be, it is a matter of record that he stood out against the chromosome theory of heredity for years after all other geneticists had accepted it, until finally convinced by the demonstrations set before him in Morgan's laboratory † ; and that

* Based on the Bateson Lecture delivered at the John Innes Horticultural Institution on Friday, 8th July 1953.

† As an example of Bateson's attitude to cytology in 1894 I cannot refrain from quoting his remark on pp. 21-22 of the *Materials*, where he briefly anticipated D'Arcy Thompson and other modern biologists in stressing the importance of a proper consideration of *pattern* in biology. " If anyone will take into his hand some complex piece of living structure . . . and will ask himself how it has come to be so, the part of the answer that he will find it

he never could bring himself to the reconciliation between the idea of continuous variation and evolution and that of discontinuous inheritance, between the essentially selectionist approach of the pure biometrician and the essentially anti-selectionist view of the pure mutationist—the reconciliation which was initiated by Morgan's young men in the '20's and was made the corner-stone of future advance in evolutionary genetics by R. A. Fisher in his great book in 1930.

Even in 1894, however, he anticipated some modern geneticists' views on selection by writing :—" while the only test of [biological] utility is the success of the organism, even this does not indicate the utility of one part of the Economy, but rather *the nett fitness of the whole* " (italics mine).

But Bateson's instinct was correct in two important respects. First and foremost, in the conditions prevailing in the biological world around the turn of the century, emphasis on discontinuity was not only desirable but necessary for progress. Without the discovery of the discontinuous or, as we now say, the particulate basis of inheritance, the biometrical approach would have remained sterile. By a strange paradox, although continuous variation and gradual evolution are the rule, they could not be properly analysed or understood except in terms of discontinuous genes and abrupt mutations.

And secondly, it was necessary to go to the facts of evolution, as revealed by the comparative study of variation in nature, to jolt the then leaders of biological thought out of their armchairs and enlarge their horizon beyond the limits of the assemblage of forms they chose to introduce into their laboratories.

Both of these aspects of his work are still extremely relevant. His belief in the evolutionary importance of markedly discontinuous variations has turned out to be justified, even though the phenomena and their explanation are very different from anything which he could have imagined at the time : and it is only by going to the facts of nature, as revealed in the natural history of all groups of animals and plants, and analysing them on a comparative basis, that we are reaching this new understanding of the importance of discontinuity.

Bateson (1894, p. 20), introduced into the study of variation the useful terms *merism* and *meristic*. I propose to introduce the term *morphism* and its derivatives, *morphic* and *morph*. I make no apology for this. Brief terms for genetic polymorphic variance are badly needed ; *form* is too general, and *phase*, though sometimes used, especially by ornithologists and mammalogists, for colour-morphs,

hard to give, is that which relates to the perfection of its pattern. And it is not only in large and tangible structures that the question arises, for the same challenge is presented in the most minute and seemingly trivial details. In the skeleton of a Diatom, . . . the scale of a Butterfly . . . *in the wreaths and stars of nuclear division* (italics mine), such patterns again and again recur, and again and again the question of their significance goes unanswered." It is only fair to add that Bateson's view of distinctive patterns or alternative forms as being what, following Galton, he called *positions of organic stability* (pp. 36, 42, 65), foreshadows many important modern ideas and discoveries concerning developmental mechanics, and helps us to understand the alternative canalisation of various morphisms (see p. 28).

has other connotations, and is, I think luckily, not generally accepted. *Polymorphism* is not merely unnecessarily lengthy, but unduly general in sense. We speak of *forms*, not *multiforms*, of a species ; and provided we define the term accurately, we can conveniently employ a brief and convenient word like *morph*.

I restrict the term *morphism* to genetic polymorphism, as defined by Ford (1945*a*), in which (usually sharply distinct) genetic variants or *morphs* coexist in temporary or permanent balance within a single interbreeding population in a single spatial region, and in such frequencies that the rarer cannot be due solely to mutation, or to the spread of selectively neutral mutants. It is important to have a term applicable in this restricted sense, since the evolutionary implications of other phenomena often loosely included under the head of polymorphism—geographical, seasonal, and all non-genetic cases, as well as mere high recombinational variance, whether with or without previous hybridisation, are very different.

In *Materials for the Study of Variation*, Bateson had already noted many cases of real or supposed genetic morphism in nature. These include the " high " and " low " forms of beetles like *Xylotrupes* and of earwigs like *Forficula* (in the latter case, he notes the geographical variation in morph-ratio ; see p. 25) : the colour-morphism of various common beetles, such as *Phratora* and *Telephorus* (which would well repay investigation by modern methods) ; of Lepidoptera such as the Clouded Yellow, *Colias* (see p. 12), the Crimson Underwing, *Catocala nupta*, and the Burnet Moth, *Zygaena* (foreshadowing the work of Bovey, 1941) ; of the egg-colour of various Copepods (curiously enough, he overlooks the mimetic egg-colour morphism of Cuckoos) ; of tibia-colour in grasshoppers, foreshadowing the work of Nabours (see below) ; of plants such as the Pimpernel, *Anagallis arvensis* (which I discuss on p. 15), the Daffodil, *Narcissus corbularia*, and other species (in flower-colour), and the Yew, *Taxus baccata*, and the Raspberry, *Rubus idaeus* (in fruit-colour) : the pattern- and colour-morphism of molluscs like the Dog-whelk, *Purpura lapillus*, the Periwinkle, *Littorina rudis*, and some species of *Chiton* (but curiously not of *Cepaea* ; see p. 9) ; of butterflies like *Hipparchia tithonus* and *Satyrus hyperanthus* ; of beetles like the Ladybirds, *Coccinella bipunctata* and *decempunctata* (as against its absence in *C. septempunctata* ; and see Dobzhansky, 1951, pp. 130, 152) ; of the butterflies *Pyrameis cardui* (in Australia) and *Terias*, and the cryptic Leaf-butterfly, *Kallima inachys* : of petal-number in various flowering plants (possibly not genetic) ; and the dextral-sinistral dimorphism of certain Gastropods.

He rightly compares the " discontinuity of sex " with discontinuous (morphic) variation in general. But he confuses the discontinuity of true genetic morphism with that of meristic variation, of gynandromorphism, of sporadic mutation, and of developmental abnormalities of various kinds.

I would here like to record my thanks to various colleagues First and foremost to Dr E. B. Ford, F.R.S., and his co-worker Dr P. M. Sheppard for their invaluable

help. Further to Sir Ronald Fisher, F.R.S., Sir Edward Salisbury, F.R.S., Dr Cyril Darlington, F.R.S., Professor Kenneth Mather, F.R.S., Professor S. C. Harland, F.R.S., Dr W. B. Turrill, Professor Hovanitz, Dr Fraser Roberts, Mr John Gilmour, Professor C. M. Yonge, F.R.S., Professor T. A. Stephenson, F.R.S., Dr Charles Elton, F.R.S., Dr David Lack, F.R.S., Dr H. N. Southern, Mr Marsden Jones, Dr A. J. Cain, Dr John Heslop Harrison, Professor Ernst Mayr, Professor T. Dobzhansky, Dr Burnett, Dr A. Allinson, Dr Ian Aird, Dr Gwynne Vevers, Mr J. Fisher, and others too numerous to mention, who have answered my queries or given me references.

In addition, I wish to pay tribute to those who, by their special contributions to the subject, have established the evolutionary importance of morphism. Bateson I have already mentioned: he was the precursor. R. A. Fisher must without doubt be acclaimed as the founder of the genetical theory of morphism, with his demonstration, twenty-six years ago, that morphism cannot exist in nature without a selective balance between the morphs concerned. E. B. Ford in his brilliant surveys has followed up Fisher's ideas and their applications, and J. B. S. Haldane has made valuable contributions to morphic theory.

Erwin Streseman in his *Mutationsstudien* first showed the importance of morphism in the micro-evolution of birds, and Ernst Mayr has extended his work and related it to modern genetical theory. Various botanical geneticists have unravelled the complexities of the special type of morphism seen in the incompatibility mechanisms of plants. Cyril Darlington has done much to demonstrate the importance and widespread occurrence of chromosomal and chromosegmental polymorphism, while Theodosius Dobzhansky, following up his penetrating study of inversion-morphism in *Drosophila*, has contributed notably to the general theory of morphism. But the subject has not always received due attention. Thus it is not specifically mentioned in Darlington's *Evolution of Genetic Systems*, in Srb and Owen's *General Genetics*, or Grüneberg (1947), though he cites several examples.

At the outset, I would like to stress the extremely widespread occurrence of morphism. Among animals, I have found reference to its occurrence in all classes of Vertebrates, and in the Urochorda, among Chordates; among Insects, in the Coleoptera, Diptera, Lepidoptera, Hymenoptera, Odonata, Dermaptera, Hemiptera, Orthoptera, and possibly Isoptera; among Arachnida, in Spiders; among Crustacea, in Copepoda, Isopoda, and Decapoda; among Echinoderms, in Asteroidea, Holothuroidea and Ophiuroidea; among Molluscs, in Gastropoda and Lamellibranchia; among Coelenterates, in Anthozoa and possibly in Leptomedusae; among Sponges, in Demospongiae; and I have doubtless missed other occurrences. Among plants, my references include cases among various Orders both of Dicotyledons and Monocotyledons, and several groups of Fungi; and I am sure botanists will know of many other cases of morphism in many other groups.

The characters involved in morphism are equally varied. They include colour, pattern, structure, sex-chromosomes, secondary sexual characters, caste in certain insects, essential oils in certain trees, reproductive incompatibility, clutch-size in birds, blood-groups, blood-diseases, chromosomal and chromosegmental variation, migratory behaviour, heterokaryosis in fungi, sensory thresholds, general habit, temperature-tolerance, disease-resistance, cancer-proneness—indeed probably every conceivable character of every developmental stage.

2. ADAPTIVE MORPHISM

After a survey such as this, the question inevitably arises why morphism is so widespread. The answer, I think, is that it provides a method of intraspecific differentiation for adapting the species to sets of sharply distinct environmental conditions. We often speak as if the environment of a species were uniform, whereas in the great majority of cases it presents marked fluctuations in all its major components—temperature, humidity, availability of food, abundance of enemies, shelter, and many other factors—both in space, from habitat to habitat and from one geographical area to another, and also in time, from day to day, from season to season, and from year to year. A species must accordingly be adapted not only to the constant factors of its environment, or to its mean conditions, but to its variations.

Adaptation to environmental variation may involve better avoidance of or greater resistance to extreme conditions, or may consist in the exploitation of a wider range of habitats or niches. In highly mobile species avoidance of extreme conditions may be effected by migration ; but greater resistance to or fuller exploitation of contrasting conditions can only be effected by greater differentiation of the species. Much of this differentiation may be non-genetic. It may be purely modificational, as in the case of plants with high plasticity ; or it may be physiological and reversible, as in the adaptive colour-changes of chameleons or flounders ; or it may be developmental (ontogenetic), as in the adaptation of larvae and adult to different conditions, or the exploitation of different environmental conditions for feeding and reproduction, as in salmon or eels ; or it may be cyclical, as in the alternation of generations in ferns, or cyclomorphosis in Cladocera, or seasonal polymorphism in butterflies, or the phases of locusts (see p. 52).

But, finally, it may be genetic. In this case, two or more distinct genetically-determined forms co-exist in balance in the population, each adapted to, and therefore enjoying differential survival in, one distinct set of conditions confronting the species, or one habitat of the many available. The result is balanced genetic polymorphism—morphism.

Morphic differentiation is able to originate so frequently (i) because so many mutants are ambivalent—*i.e.* have both selectively favourable and unfavourable effects (see p. 25) ; (ii) because inversion and translocation provide the gene-complex with sets of distinct " supergenes " incapable of crossing-over and therefore capable of independent evolution in different adaptive directions ; (iii) because variations in chromosome-number and -type may persist within a population, as with supernumeraries in plants, and as in chromosomal sex-determination ; (iv) because some genetically-determined differences produce sharp meristic discontinuity (*e.g.* in egg-number in birds) ; (v) because in certain circumstances extremely high variability is an asset. In any case, the sharply distinct environmental conditions with which most organisms are confronted may be better met by the

sets of sharply distinct characters provided by morphism than by unimodal adaptation to the mean conditions. Morphism thus frequently serves as an adaptation to the extremes of environmental variation.

At the outset, we must make a distinction between *phaneromorphism*, in which the morphs are *manifest* or readily distinguishable by inspection, and *cryptomorphism*, in which they are not; and also between the phenotypic *phenomorphs* and the *genomorphs* which are their genetic basis. From the genetic or genomorphic point of view, we may distinguish *genic* morphism, in which the differences between morphs depend on one or a few single genes; *continuous* morphism (p. 20), where so many genes are involved that the sharp distinctness of the morphs is submerged in excessive continuous variability, *chromosegmental* morphism, in which the differences between morphs depend on sections of chromosomes; and *meristic* morphism, in which they depend on genetic thresholds determining a series of large meristic characters.

I will begin with the most interesting type of morphic adaptation, adaptive genic phaneromorphism. An obvious example is the mimetic morphism of butterflies (Ford, 1953; Carpenter, 1925; Eltringham, 1910; Beebe, 1955; Goldschmidt (1945), whose anti-selectionist review of the subject, however, is very one-sided). It is an advantage for a Batesian mimic to have more than one model, for this spreads the risk and increases the potential total population; but clearly an appearance intermediate between two sharply distinct models would be of no selective value. Some such species have two, others three or more mimetic morphs. The selective value of the resemblance is shown by the way in which the geographical variation of the models is followed by that of the mimetic morphs, and conversely by the breakdown of the resemblance in areas where the model is absent or rarer than the mimic.

In some species non-mimetic as well as mimetic morphs are found. This indicates a general tendency to morphism. Any resemblance of some of the original morphs to a nauseous species would then be improved by natural selection.

A further point of interest is that in the great majority of cases, mimetic morphism is confined to the female sex, the males being non-mimetic. Ford suggests that this is due to sex-recognition being dependent on the visual recognition of males by females.

Genetically, the appearance of the mimetic morphs depends on a large number of genes, all controlled by a single switch-gene which is in selective balance with the other main morphic gene or genes. If a morphism is of adaptive value to the species, it is important that the morphic genes concerned shall be preserved in balanced co-existence within the population, and that none shall be lost to the species by differential adverse selection in extreme conditions. In mimetic morphism, this balance could theoretically be maintained by variations in external selection-pressure, for the selective advantage enjoyed by a mimetic morph decreases as its abundance increases,

and vice versa (Ford, op. cit.). However, in practice, the balance appears to be also secured genetically, the homozygous dominants being at a marked selective disadvantage, and the heterozygotes enjoying a selective advantage.

This heterotic type of genetic stabilising mechanism, in which the co-existence of morphic partners is maintained by means of some intrinsic selective advantage enjoyed by the heterozygote, is found in most morphisms, but in various forms. In genic morphism, the balance is usually preserved by a mechanism which I shall call *double-dose disadvantage*. This is preferable to *over-dominance* (Crow, 1952 ; Hull, 1952), as it is unrelated to dominance in the accepted sense. Alternatively the term *dosage heterosis* might serve. One of each morphic pair then depends on a dominant mutant, which in single dose confers a selective advantage over the recessive (normal) type, but when in double dose is either lethal, sublethal, or, to use a term to denote slightly subnormal general fitness or viability, subviable. In such cases, the favouring of the heterozygote does not depend on an advantage due to increase of " vigour " or fitness arising from the fact of its heterozygosity, but from the advantage of the mutant over the normal when in single dose, combined with its disadvantageous subviability when in double dose ; (see also p. 50).

When genic polymorphism occurs, all the morphic genes except one usually show double-dose disadvantage, so that we have a so-called " universal recessive " together with a number of dominant morphs.

Since it is advantageous to make the balance mechanism as strong as possible, any other mutant in loci close to the primary morphic gene and possessing both double-dose disadvantage and either single-dose advantage or neutrality will tend to be preserved. Further, as R. A. Fisher pointed out many years ago (1930), selection will automatically operate to produce closer linkage between all mutants concerned with a given morphism, which happen to arise in the same chromosome. And finally, as Ford has stressed, genes with strong double-dose disadvantage will provide genetic shelter to any recessive lethal mutations which happen to arise close to them, and the recessive lethals will then increase the double-dose disadvantage of the system. Thus in all these ways, a morphic balance-mechanism which originally consisted of a single gene-pair with double-dose disadvantage will tend to be converted into a system of closely linked genes whose total double-dose disadvantage is much stronger, and its stability or resistance to disruption therefore much increased.

In all cases of mimetic morphism in butterflies so far investigated, double-dose disadvantage is operative. However, much more work is needed before the genetic situation is fully clarified.

A somewhat similar example is that of the egg-mimicry of the Cuckoo, *Cuculus canorus* (Southern, 1954). Here the mimetic resemblance reduces the risk of the host-parents ejecting the brood-parasite's egg, while the polymorphism provides a greater range of hosts and so permits a larger total population to exist. However,

the Cuckoo's morphic egg-mimicry differs from the adult morphic mimicry of butterflies in various ways :—(1) intermediate types are more frequent ; (2) in some areas such intermediate egg-types are much rarer, and the mimetic resemblances to host eggs are much closer ; (3) whereas in the butterflies segregation within one brood is no disadvantage, in the Cuckoo it is essential that each female should produce only one egg-type.

Southern considers that the variance here is intermediate between geographical differentiation and true balanced morphism, leading to the production of partially isolated strains or *gentes*. Where large areas of distinctive habitat exist (*e.g.* forests, reed-beds, open plains) the local gentes can develop a high degree of egg-mimicry for one or a few host species ; but where human interference (or other agency) has broken down the habitat areas into smaller blocks and intermingled them, as in Western Europe, the gene-systems underlying the different mimetic egg-patterns are also largely broken down through intercrossing. This implies that major switch-genes are not operative. It is not known whether double-dose disadvantage occurs.

Like various mimetic butterflies, the Cuckoo shows a general tendency to morphism (Voipio, 1953), for it shows a plumage dimorphism, barred red and unbarred grey. Among adults, the red morph is confined to a minority, all females ; among juveniles, it occurs in both sexes, but in a majority of females and a minority of males. Voipio suggests that there is a morphic rate-gene concerned with the deposition of (grey) eumelanin, which has a lower operative threshold in males, so that all adult and most juvenile males are grey. Furthermore, the distinction between the adult morphs is sharp, whereas in juveniles, presumably owing to their being in different stages of eumelanin production, there are numerous intermediates. Voipio further considers that the plumage-morphism is mimetic, the dorsal view of the unbarred grey morph resembling the Sparrowhawk, that of the barred red morph the Kestrel and Merlin. The advantage accruing to the red female morph from these resemblances lies, he suggests, in the grey male being chased away by fosterers because of its resemblance to a Sparrowhawk, leaving the coast clear for the female. In support of this he adduces some figures indicating that the frequency of the red adult morph is higher in wooded habitats, where Sparrowhawks are common. The red adult female morph also occurs in *C. saturatus* and *C. poliocephalus* from the S. Palearctic, but more commonly than in *C. canorus* ; these also are predominantly parasites of woodland host species. Many other cuckoos show egg-mimicry (Baker, 1942). Dr A. H. Sturtevant informs me that some species of *Volucella* are morphic, like some of its bee hosts. This may prove to be a mimetic morphism.

Morphic egg-mimicry could presumably only arise in species whose egg-colouration is already polymorphic or highly variable. It is worth recalling that some birds, such as the Guillemot, *Uria aalge*, the Red-backed Shrike, *Lanius collurio*, and the Tree-Pipit, *Anthus*

trivialis, show such non-mimetic egg-morphism to a much greater extent than related species. A comparative study of morphic egg-variation in birds would be of considerable interest.

The primary morphism in such cases depends on a balance of viability (intrinsic general fitness) factors. A similar secondary extrinsic adaptive utilisation of such a primary viability-morphism is to be seen in the snails of the genus *Cepaea*. Here the manifest colour and banding morphs appear to have originally been " correlated characters " in Darwin's sense—non-adaptive phenotypic effects of the morphic viability genes ; but Cain and Sheppard (1950) and Goodhart (1954) have shown that they now collectively confer cryptic protection from predators, notably song-thrushes but also rabbits, over a wider range of habitats than would be possible without morphism. Thus unbanded browns are at a selective advantage in beechwoods, banded non-browns in hedgerows.

The similar morphism of the Garden Snail, *Helix hortensis*, is largely masked by uniform dark brown pigment, but Sheppard (*in litt.*) tells me that even so there is evidence of the adaptive (cryptic) value of morphism in general tint or shade in relation to variations of tint or shade of soil-colour.

Reproductive morphism may be highly adaptive. Thus the Swift, *Apus apus* (Lack, 1947-48, 1954), is dimorphic as regards clutch-size, females laying either two or three eggs. In favourable seasons the three-egg females produce a higher absolute number of fledged young than the two-egg females, but in cold and wet seasons a lower absolute number, since the smaller amount of insect food is less adequate for three than for two nestlings. The balanced coexistence of three-egg and two-egg reproductive morphs is thus an adaptation, acting as an insurance against the regular occurrence of bad seasons.

At Oxford, the two-egg morph is rather commoner, but the proportion of the two morphs varies geographically, the three-egg morph becoming somewhat more abundant in higher latitudes, where longer day-length ensures a greater total time for catching food. Such a graded geographical alteration in proportion of morphs constitutes a *ratio-cline*, and characterises many morphic systems.

Similar but usually more striking ratio-clines in egg-number morphs (which are most easily detected as a graded geographical change in mean clutch-size) occur in a great many birds (Lack, 1947-48). In any given locality, as would be expected, there are distinct egg-number morphs (Lack, 1953, Kluiver, 1951). This type of adaptive reproductive morphism thus appears to be widespread.*

While in such cases there obviously must be a genetic basis for the tendency to lay more or fewer eggs, the *discontinuity* between the

* Some species are " determinate " layers, like the Herring Gull, *Larus argentatus*, normally monomorphic for a clutch of three (Tinbergen, 1953), and thus have foregone the adaptive insurance provided by egg-number morphism. Other species, again, like the Short-eared Owl, *Asio flammeus*, have a physiological instead of a genetic mechanism for adjusting egg-number, and can markedly increase or decrease clutch-size in immediate reaction to available food-supply.

morphs is not genetic but meristic, depending on the fact that a bird's clutch of eggs consist of a few large discontinuous units. If the range of egg-number in Swifts were from 200 to 300 instead of from two to three, a sharply bimodal morphism in clutch-size could not arise, except with the aid of some special genetic switch-mechanism. It is interesting to speculate at what absolute number this failure of meristic discontinuity to provide a basis for effective morphic discontinuity would set in : I would hazard a clutch-size of between ten and twenty.

A precisely analogous reproductive insurance is provided by the morphism of " hard " and " soft " seeds in various plants, " soft " seeds germinating immediately, " hard " seeds only after considerable delay (see Crocker and Barton, 1952). The phenomenon is well-known, but has not been scientifically investigated save in a few cases. *E.g.* in the Rockrose, *Helianthenum guttatum*, Juby and Pheasant (1933) find that a single plant may produce some seeds germinating after a few days, others only after much longer periods, which would delay growth until the following spring, and thus be of advantage in severe winters. The difference in germination is determined by variation in the permeability of the seed-coat. The " hard " seeds show a multimodal frequency for their germination-time, indicating that several genes are operative ; while failure to obtain strains pure for any particular germination-delay implies the existence of a genetic balance-mechanism underlying the morphism.

In Nicandra, germination-time morphism depends on the number of isochromosomes present (Darlington and Janaki-Ammal, 1945). A minority of plants have one instead of the more normal two isochromosomes. One-isochromosome seeds show delayed germination. Darlington has suggested to me that the variation of many plants in supernumerary or B-chromosome number, provides a balanced switch-mechanism for morphic adaptation in germination-date, drought-resistance, etc. The grasshopper, *Melanoplus differentialis*, shows egg-dimorphism, one morph developing directly, the other with an obligatory diapause (Bodine ; Bodine and Boell ; H. C. Burdick). Further investigation would be profitable.

A similar insurance mechanism is provided by the morphism in migratory behaviour of many birds (Lack, 1943). Single populations (and even single broods (Nice, 1937, 1943, *Trans. Linn. Soc. N.Y.*, *4*, *6*)) contain individuals which remain resident throughout the winter, and others which develop the migratory urge. Further, there are well-marked ratio-clines, the percentage of non-migrators often falling to zero in the north, and rising to 100 per cent. in the southern parts of the birds' range.

In most areas there is a simple dimorphism between migrators and non-migrators. In Britain, however, a trimorphism has been evolved since the end of the last glacial period, there being two quite distinct migrator morphs, the presumably original southward migrators and those which migrate westward to the mild climate of western

Ireland. Migratory morphism is also known in song-sparrows and other North American birds, and presumably occurs in all migratory Passerines and Limicolines.

My examples so far have all been phaneromorphic : however, cryptomorphism also can be highly adaptive. Cryptomorphism was discovered by Dobzhansky in *Drosophila* (see his admirable summary, 1951, Ch. V and VI). Interestingly, this large genus shows only one or two phaneromorphisms (*e.g.* the abdomen pattern of *D. polymorpha* ; de Cunha, 1949, 1953). However, many, perhaps most, species are morphic for chromosegmental inversions, which are detectable by inspection of salivary gland preparations. Inversion prevents crossing-over between the chromosegments concerned, so that the inverted and " normal " chromosegments can behave as supergenes in Darlington's sense. Cordeiro and Dobzhansky (1954, *Amer. Nat.*, *88*, 75) find that all " wild " chromosomes differ in selective value, both when homozygous and in various heterozygous combinations.

In all cases investigated, the two chromosegmental morphs of each such inversion-morphism have evolved divergently so as to adapt their possessors to two sharply alternative conditions—summer and winter, low and high altitudes, urban and rural habitats, favourable and unfavourable years, etc. Thus *D. pseudoobscura* in California contains third chromosomes with morphic chromosegments, called *Standard* and *Chiricahua*. In the high temperatures of summer the frequency of Standard decreases, while that of Chiricahua increases, and conversely in the lower temperatures of spring and autumn. If the population were pure for Standard, it might be unable to survive exceptionally hot summers, and vice versa. In order to prevent the elimination of one or other temperature-adapted chromosegment in exceptional seasons, the two alternative chromosome-morphs are automatically tied together in a balanced morphic system : the heterozygous combinations enjoy a marked selective advantage, and this prevents the elimination of either member. This *euheterosis*, to use Dobzhansky's useful term, is not a necessary consequence of the heterozygosity of inversions, for it is not found when inversions from distant areas are brought together and combined artificially. It must therefore have been evolved co-adaptively as a stability mechanism for securing the permanence of the morphic system. Inversion morphisms have been detected microscopically in various other Diptera (White, 1954).

Inversion (chromosegmental) morphism thus differs from genic morphism in several interesting ways. First, the permanence of the balance-mechanism is provided for by a specially evolved euheterotic co-adaptive system of genes in the two chromosegmental partners, conferring on the heterozygotes a marked positive selective advantage in respect of viability or other aspect of intrinsic general fitness ; whereas in genic morphism the same result is obtained, but by the different method of double-dose disadvantage. Secondly, whereas

euheterotic advantage has to be evolved, some degree of double-dose disadvantage appears to be present from the start in genic morphisms, though it may be strengthened later (see above). Further, whereas in most genic morphisms one allele of a morphic pair is a simple dominant and there are thus only two distinct phenomorphs, in inversion morphism neither supergene is dominant and there are three phenomorphs—the two homozygotes, each adapted to one of two contrasting environmental conditions, and the heterozygote adaptively equipped with an advantage in general fitness (p. 27).

The efficiency of such a system in securing an equilibrium between the morphs concerned has been experimentally demonstrated by Dobzhansky. When different proportions of two inversion-morphs are used to set up a number of breeding populations, their frequencies will change, tending after about ten months to a certain constant ratio. The precise final ratio will vary with varying conditions of temperature, food, etc., but in any one set of conditions the final ratio is the same, whatever the ratio at the beginning of the experiment.

As would be expected from these facts, both altitudinal and geographical ratio-clines for inversion-morphs occur in nature. The geographical ratio-clines present a rather complicated picture. It is significant that in different *Drosophila* species, wide range and biological success are correlated with abundance of inversion-morphisms.

White (1954) cites numerous cases of morphism in chromosome-number, but their underlying stability-mechanisms are mostly uninvestigated and their selective implications unknown.

The morphism exhibited by the Clouded Yellow butterflies, *Colias*, is of exceptional interest since it has been studied in all its aspects—taxonomic, genetic, ecological, and evolutionary (Hovanitz, 1953; Komai and Aé, 1953; and see Ford, 1945). Most species of the genus are monomorphic yellow in the male sex, but dimorphic yellow and white in the female. The monomorphism of the males is presumably due to the need for a uniform sign-stimulus for sex-recognition, as in polymorphic mimicry (see above). The white morph is determined by a single dominant allele, with sex-limited manifest effect. This is closely linked with a "recessive lethal"—*i.e.* a gene with extreme double-dose disadvantage, so that crosses give a 2 : 1 instead of a 3 : 1 ratio in F2. This gene presumably enjoys some single-dose advantage, as it is present in all wild populations in the same frequency as the white allele. It is to be expected that the white allele itself has some double-dose disadvantage, but this has not yet been proved. However, since monomorphic white populations occur near the northern limit of the range of some species, its linkage with the lethal can be broken naturally as well as experimentally, and any double-dose disadvantage of white must be small enough to be capable of being buffered by compensatory modifiers.*

* In the Japanese subspecies of *C. hyale*, Komai and Aé (1953) find only a probable small subviability of homozygous whites, indicating that the adjacent recessive lethal is not present; but a considerable deficiency of homozygous yellows occurs.

The two morphs differ in their behaviour with regard to temperature, the whites being more active at lower temperatures, so that in California they fly (and oviposit) mainly in the mornings and evenings, the yellows mainly in the midday hours. This behaviour-difference is reflected in corresponding ratio-clines, both altitudinal and geographical. Geographically, white frequency increases with north latitude, or rather with decrease of temperature, but the precise form of the cline differs in different species of the genus, showing that the basic behaviour-difference has been quantitatively modified in the course of speciation.

The adaptive value of the morphism clearly consists in increasing the range of environmental conditions and habitats which can be profitably occupied, both by any given population and by the species as a whole. However, this fact would probably never have been discovered if the biologically important character of differential temperature-reaction had not been associated with the manifestly visible but biologically unimportant "correlated character" of colour-morphism. The classical case of female dimorphism in the Silver-washed Fritillary, *Argynnis paphia*, is similar in genetic basis and possibly in behavioural result, but differs in being geographically localised (Ford, 1945*b*).

The existence of all-white populations in the northern parts of the range of various species shows that a primary dimorphism shared by many or all species of a genus may give rise to *secondarily monomorphic* populations. If these then become isolated from the rest of the species, they could readily differentiate to the subspecific or specific level. Such secondarily monomorphic groups would then constitute a hitherto little-recognised category of taxonomic units, produced by a peculiar mode of evolution.

We have already met with secondary monomorphism in migratory birds. It occurs also in many other groups: a classical example is the Australian Goshawk, *Accipiter novae-hollandiae* (Southern and Serventy, 1947; Stresemann, 1926). This species is dimorphic, with white and dark morphs in very variable frequency. Near the southern boundary of Queensland about 80 per cent. darks occur; from here an irregular double ratio-cline extends along the coast, northwards to New Guinea (where the picture is complicated by the presence of a different dark allele, producing rufous or brown instead of grey plumage, and by a separate dimorphism in juvenile plumage: Mayr, 1940), and round to the north coast of Western Australia, and southwards to Tasmania. In New Guinea, whites are moderately frequent, but totally absent on some islands, presumably due to their colonisation by dark birds only. In Tasmania the entire population is secondarily monomorphic white, possibly because whites are favoured by humidity.

In this case the secondarily monomorphic white population has not undergone further taxonomic differentiation as a result of its isolation on an island. But in the dimorphic Great Blue Heron of

America, *Ardea herodias*, the white morph shows an increasing ratio-cline eastward from Yucatan to culminate in 100 per cent. white on the Florida Keys. This all-white population has usually been separated as a distinct species, *A. occidentalis*, but is almost certainly a subspecific specialisation of a secondarily monomorphic population of *A. herodias* (E. Mayr, 1955).

Mayr (1942, p. 78 ff.) cites other examples. However, only that of the Lory, *Charmosyna papou*, in which three of four subspecies are dimorphic for melanism but one is monomorphic and lacks the melanic morph, would seem to illustrate this process. None of the others are morphic in any single population; they merely show subspecific divergence in respect of sharply distinct characters. In the Bulbul, *Microcelis leucocephalus*, this is further demonstrated by the existence of hybrid zones, containing " a medley of hybrid forms " (recombinations), where pairs of the very distinctively plumaged subspecies have come secondarily into contact. Such facts are a salutary warning not to confuse the recombinational polymorphism due to secondary hybridisation with true balanced morphism. Another case where polymorphism is probably due to hybridisation is the African Barbet, *Lybius torquatus* (Mayr, 1940, p. 77).

Secondary monomorphism is found also in some Pacific land-snails, such as *Partula otaheitana* in Tahiti (Crampton, 1932). This species is dimorphic as regards direction of coiling, but monomorphic dextral and monomorphic sinistral microraces occur. Such secondary monomorphism, which is also found in respect of colour and pattern in forms like *P. clara*, *suturalis* and *mirabilis*, which are expanding their range in a region with many small habitat-areas, is to be expected as the result of random sampling during the colonisation of new areas. For similar phenomena in other oceanic land-snails see Huxley, 1932, pp. 232-4; and in flatfish, etc., p. 50 of this paper.

A peculiar picture is presented by the eastern palearctic lady-beetle, *Harmonia axyridis* (see Dobzhansky, 1951, p. 142). In the eastern parts of its range this species is highly morphic in colour and pattern, with the yellow morph increasing in frequency eastward. In its western area, however, it is virtually monomorphic for one particular colour-pattern; most other morphs were wholly absent, but two occurred in a total of over 4000 individuals examined, and must therefore be regarded as not in morphic balance, but as rare mutants.

The common squirrel of Europe, *Sciurus vulgaris*, is dimorphic red and black. The British form, however, is monomorphic red, and has undergone subspecific differentiation (Matthews, 1952). The American Grey Squirrel, *S. carolinensis*, has a melanic morph, which in some areas once exceeded 50 per cent.; but the ratio-cline needs fuller analysis (Shorten, 1954). The dimorphic Arctic Fox, *Alopex lagopus*, is monomorphic white in Kamchatka and blue on some Alaskan islands (see Huxley, 1942, p. 103). I found Voipio's important paper (1950) too late to include his valuable facts on Squirrels, Foxes,

Hares, and Musk-rats : the spread of the apparently deleterious *Samson* mutant of the Red Fox to become an established morph in areas of dense human population is especially interesting.

In plants, the common Scarlet Pimpernel, *Anagallis arvensis*, has flowers " usually red or pink, more rarely blue or lilac " (Clapham, Tutin and Warburg, 1952, p. 809), a tetramorphism presumably due to two morphic gene-pairs. However, the form *A. a. foemina*, classed as a distinct subspecies and distinguished by minor differences in length of pedicel and calyx-teeth, and shape and hairiness of corolla-lobes, is monomorphic blue-flowered. In Britain it is rare and confined to the south and west, so that it has probably differentiated as an ecogeographical subspecies.

The rapidity with which selective balance may be altered and one morphic allele may replace its partner in certain conditions is beautifully illustrated by the facts of industrial melanism in moths (Ford, 1945, 1955). In the past 100 years, in the industrial areas of Britain and NW. Europe, forty-eight species of moth have become full melanics and some 250 others have become markedly darker. In *Biston betularia*, Haldane (1923) calculated that the coefficient of selective disadvantage of non-melanics in industrial surroundings is at least 0·33, which implies an astonishingly high intensity of selection.

Ford has shown that all industrial melanism is due to the spread of dominant genes, which occur regularly as rare mutants in normal (non-industrial) habitats : no recessive melanic mutants have spread. The selective balance is between the cryptic value of normal colouration, which is very high in rural areas but lower in the darker environment and the lower predator-frequency of industrial areas ; and the greater hardiness of dominant melanics and their high resistance to unfavourable conditions such as soot- and smoke-contaminated food.

In most species, the dimorphism in any one area is transient ; in the course of a few decades the population is converted from an overwhelmingly non-melanic to an overwhelmingly melanic one. The species as a whole thus comes to show what can be called geographical morphism ; but this is only a special case of geographical differentiation, differing from the usual process seen in wild species merely in its greater rapidity and in the fact that only one gene with major effect is involved, instead of many genes with small or moderate effects. However, there are indications of double-dose disadvantage in some melanics, with the consequent establishment of a true balanced morphism in certain areas (Kettlewell, *in litt.*).

I may here mention other cases of morphic melanism, though the adaptive value of the black morph is not always known.

Thus the melanic morph of the fur-bearing mammal the Hamster, *Cricetus cricetus*, has increased in frequency and extended in range during the past 150 years in SW. Russia, until now in four sub-steppe (woodland steppe) areas about 25 per cent. of pelts are

black. These " black spots " are surrounded by areas of decreasing melanic frequency, until in the true steppes melanics occur only as rare mutants (Gershenson, 1945 ; and see Huxley, 1942, p. 103, and map in Dobzhansky, 1951, p. 143). The melanic morph appears to be favoured by moist conditions, and its spread must have been facilitated by alterations produced by human interference.

The Brush Opossum, *Trichosurus vulpecula*, is monomorphic grey in mainland Australia, but in Tasmania is dimorphic, with melanics up to 100 per cent. in some areas (Guiler, 1953 ; and see Huxley, 1939). Guiler finds that high melanic frequency is favoured by a combination of high rainfall and dense forest cover ; where man has abolished the forest cover, a sharp fall in melanic frequency occurs. The populations of the small islands between Tasmania and the mainland show no morphism (see p. 51, and Pearson, 1938).

Melanic morphism also occurs in the Rabbit, *Oryctolagus cuniculus*, in Tasmania, but also, unlike the Brush Opossum, in wet areas of the Australian mainland (Barber, 1954). The melanics here appear to be at a cryptic disadvantage against predators when in open country, which is not the case with the black Brush Opossums. (In Tasmania, the melanic morph in the Brush Opossum appears to have reached equilibrium, but in the Rabbit appears to be still spreading. The maximum frequency of melanics is about 35 per cent., reached in the high forested plateau of the interior. As the rabbit was only introduced within the last 150 years, the spread of its melanism has been nearly as explosive as that of moths in industrial areas : Barber considers that it has taken place within the last fifty years.

It should be mentioned that on small islands off the British coast, melanic (and other mutant) rabbits are more abundant than on the mainland, though never constituting a balanced morphism. Thus R. M. Lockley (*in litt.*) found 0·5 per cent. melanic, 0·25 per cent. particoloured and 0·5 per cent. long-haired specimens among 8148 rabbits trapped on Skokholm (Pembs).

There is a rough ratio-cline in the Common Squirrel, *Sciurus vulgaris*, in Eurasia, the frequency of melanics increasing from zero in the north to 100 per cent. in the south (Matthews, 1952, p. 201, Shorten, 1954). For other mammalian cases, see p. 51.

Similar melanic morphisms also occur in birds : *e.g.* the West Indian Sugarbird, *Coereba flaveola*, the New Zealand Pied Fantail, *Rhipidura flabellifera*, and the New Guinea Lory, *Charmosyna papou* (Mayr, 1942 ; Huxley, 1955), but here, as in Squirrels, the precise climatic conditions favouring melanics have not yet been fully analysed.

Some remarkable cases of adaptive morphism occurs in West Australian frogs (Main, 1954). *Crinia georgiana* is highly polymorphic both as tadpole and as adult, and in respect both of colour (mottled, fawn, grey and red-brown) and of skin-texture (smooth, warty, ridged, " lyrate "). All ridge-skinned forms so far collected are fawn and no lyrates are red-brown, but smooth and warty may be of any colour.

The colour-morphism seems to serve as cryptic adaptation in different backgrounds, and smooth-skinned adults are less resistant to drought, and have a lower frequency in dry habitats. The smooth-skinned forms metamorphose earlier than the three " rough " forms, indicating that thyroid activity is involved. In *Crinia signifera* adults are trimorphic for skin-character—rough, smooth, and with longitudinal ridges. All three morphs occur in areas where there are swamps but only the rough-skinned form in hot dry habitats. In genetic experiments marked deficiencies from expectation occur in certain classes. Some populations appear to consist wholly of heterozygotes. *C. glauerti* appears to have a similar morphism. *C. leai* is monomorphic smooth, but shows adult colour-morphism, while *C. rosea* is wholly monomorphic.

In *Helioporus pelobatoides* the tadpoles are dimorphic, fast-developing " normals " capable of colour-change, and slow-developing " pales " which remain pale in all conditions of background or illumination, though pituitary injection will artificially darken them. The pales thus appear to be suffering from a pituitary disadvantage which affects both their capacity for colour-change and their rate of development. The counteracting advantage enjoyed by the pale morph may possibly be a skin-character rendering adults more resistant to desiccation. J. A. Moore (1943), Goin (1947, 1950) and Moriwaki (1953) also report morphisms in various Anura.

What appears to be an analogous case of morphism related to climatic resistance is found in the large Australian land-snail, *Bothriembryon bulla* (Main and Carrigy, 1953). Near Perth, W. Australia, this has a melanic morph which differs from the normal not only in its black body-colour but in the darker ground-colour of its striped shell. In one type of habitat, where the shrub, *Jacksonia gracilis*, resists fire and provides good cover for snails even after burning, the melanic morph is present but rare (5-10 per cent.) ; but in another where the ground is black and bare and there are no " refuges " provided by *Jacksonia*, the population is 100 per cent. melanic. Main ascribes the advantage of the melanic in this habitat to cryptic protection from the snail-eating Western Magpie, *Gymnorhina dorsalis*.

The British pyralid moth, *Acentropus niveus*, is unique in having two female morphs, one normal and resembling the male, the other aquatic and flightless, which protrudes its posterior end through the surface-film to permit copulation (Beirre, 1952, p. 72 ; Meisenheimer, 1921, fig. 678). The selective advantage of the aquatic female morph apparently consists in its ability to oviposit at depths far beyond the reach of the flying morph, thus extending the ecological range of the larvæ, which feed on submerged plants. Several species of water-beetle are dimorphic for flight and flightlessness (Jackson, 1952, 1955). This demands thorough investigation. (See also p. 51.)

The Plumose Sea-Anemone, *Metridium senile*, shows a high degree of morphism, apparently based on three allelic or closely linked dominants and a universal recessive, determining different biochemical

properties of the pigments involved in its colouration (Fox and Pantin, 1941 ; North and Pantin, unpubl.). The different phenomorphs have different light-sensitivities, and are adapted to different conditions of illumination. Dr Pantin informs me that the New Zealand species of *Metridium* appears to be monomorphic white, and lives in low light-intensities. A comparative study of morphism in the genus should be rewarding. A similar adaptation appears to exist in *Tealia*, but here there is also a cryptic polymorphism, differently coloured morphs resembling different backgrounds. In *Sagartia* some of the numerous morphs appear to be procryptic, others anticryptic, and still others aposematic—a remarkable spread of adaptations secured by one morphic system (Walton, 1911 ; Fleure and Walton, 1907 ; and see Stephenson, 1928-35, for these and other examples).

The orchid, *Epipactis helleborine*, is trimorphic in flower-colour, the pale violet-veined form being apparently a heterozygote between the purple and the white green-veined morphs. Weijer (1952) maintains that whites are selectively favoured in more exposed habitats. Further analysis of this species would be valuable.

In the Poecilid fish, *Lebistes reticulatus*, there is an extremely high sex-limited polymorphism, the adult males having a great variety of striking patterns while the females and immature males are monomorphic dull-coloured (Haskins and Haskins, 1950, 1951). The selective advantage of the colour-patterns is unknown. They appear not to confer epigamic advantage, since in display they are masked by a special temporary display-pattern (Baerends, 1955). They are disadvantageous in exposing their possessors to predator attack. C. P. Haskins (*in litt.*) tells me that where the dangerous predator fish *Crenicichla* occurs, the *Lebistes* population includes fewer striking colour-morphs. The different colour-morphs appear to enjoy little differential selective advantage *inter se*, artificial introductions of non-indigenous morphs into a wild population remaining at a constant ratio through several seasons. All colour-morphs depend on more or less completely dominant genes, and there is no " universal recessive " among the males (though from the evolutionary standpoint the female colouration could perhaps be regarded as such).

It is good to know that this striking morphism is being carefully studied in all its aspects, as are the somewhat similar cases of *Platypoecilus* and *Xiphophorus* (Gordon, 1948 ; Gordon and Gordon, 1950, 1954).

In *Platypoecilus* the remarkable fact has emerged that the dominant morphic genes concerned with macromelanophore patterns produces melanotic tumours in F1 or F2 from crosses with the related fish *Xiphophorus helleri*. The genes concerned with micromelanophore distribution have no such effect. It may perhaps be suggested that in *Platypoecilus* all macromelanophore morphic genes have deleterious tumour-producing tendencies as well as selective advantages and that their disadvantageous effects have been buffered by modifiers, but that the buffering system is broken down by crossing. (See p. 51.)

The bee *Melipona* is unique among Hymenoptera in apparently

possessing a genetic caste-morphism, workers and queens being produced in a ratio of 3 : 1 in *M. marginata* (Kerr, 1950). Kerr explains the facts on the basis of two gene-pairs, the queens being always heterozygous for both, the workers always homozygous. In other species the ratio is 7 : 1, which Kerr ascribes to the involvement of three instead of two gene-pairs in the determination. Kerr states that the worker : queen ratios are constant, but his figures show a frequent deficiency of queens below the expected 25 per cent. in *M. marginata*. This interesting case should be further investigated. A genetic caste-morphism of this sort is clearly adaptive, but is not so flexible as the modificational determination through differential feeding found in *Apis*.

Some authorities believe that castes are genetically determined in termites, but this has not yet been proved.

Some fungi, such as *Penicillium*, possess a unique type of morphism based on heterokaryosis, or the co-existence of two genetically different types of nuclei in a single hypha, brought about by hyphal fusion and producing positive heterosis (Jinks, 1952 ; Pontecorvo, 1946). The frequency of the two nuclear types changes markedly with conditions, thus providing a flexible adjustment to environmental change. The primary sexual dimorphism (sex-ratio) of the eelworm, *Heterodera rostochiensis*, can also be adaptively adjusted to conditions (Ellenby, 1954), though the mechanism of adjustment is not known.

Adaptive morphisms involving reproductive incompatibility are widespread, the most important being sexual dimorphism in respect of sex-chromosomes. The sex-chromosomes also provide a switch-mechanism which directly or indirectly controls the appearance of all sex-limited and secondary sexual characters, and thus permits further adaptive differentiation of the two morphs.

Other incompatibility morphisms have the adaptive function of promoting outcrossing between different strains, and so increasing the species' reserve of variance. They include the visible barriers of heterostyly as in *Primula* and *Lythrum*, and the invisible ones of pollen-style incompatibility as in *Nicotiana*, *Trifolium*, cherries, etc. In the latter type, the morphic system may be very complex, involving up to forty alleles (or pseudo-alleles held together by very close linkage).

In the heterostyly of *Primula*, the genetic mechanism is of an unusual type. Instead of the usual method involving a basic switch-mechanism and a dependent canalisation-mechanism consisting of a co-adapted collection of genes in other parts of the gene-complex, there is a multiple system, consisting of a group of closely-linked genes, each controlling one of the different developmental processes involved in heterostyly, such as stamen-height and stigma-length, and together constituting the genetic switch (Ernst, 1936; Mather, 1950; and p. 52).

As showing the delicacy of the selective balance involved, I should mention the fact discovered by Crosby (1949), that in certain areas a homostyle mutant of the Primrose, *Primula vulgaris*, may spread, so establishing a transient dimorphism of homostyle and heterostyle

forms, and leading eventually to a local geographical dimorphism. The homostyles enjoy the selective advantage of being self-compatible and capable of self-fertilisation, but suffer a considerable selective disadvantage in respect of viability.

Uphof (1938) has given a review of the occurrence of cleistogamy in flowering plants. Cleistogamy is often a matter of ontogenetic differentiation, but in some cases it would appear to be morphic, genetically-determined cleistogamous and normal plants co-existing in single populations. The selective balance is then between the long-term advantages of cross-pollination and the immediate advantages of self-pollination. However, Uphof's analysis is not very clear, and the problem demands intensive investigation.

I have given enough examples of adaptive morphism to demonstrate its widespread occurrence. It remains to mention one or two special cases.

In the Sea Plantain, *Plantago maritima*, what from one angle is obviously a morphic adaptation lacks one of the criteria by which I defined morphism, namely discontinuity between the morphs (Gregor, 1938, 1939). Here a large range of forms occurs, differing in important characters such as spike-length and scape-length, the latter for instance ranging from just over 20 to nearly 50 cm. within a single population. The different forms appear to be closely adapted to the graded conditions of the sea-shore zone transitional between water-logged mud (short scapes) and dry rock (long scapes), and in nature the different forms show a ratio-cline in forms across this zone, even when its extent is only a matter of a few metres.

It appears that the species has a high genetic variance in respect of the characters involved, and that the cline results from the differential survival of those variants best adapted to the different parts of the ecologically graded habitat. The frequencies of the different morphs are thus automatically adjusted by extrinsic selection in each generation, and the only intrinsic genetic requirement is a high variance; for whereas most adaptive morphisms are adaptations to sharply distinct environmental conditions, the environment here shows the unusual feature of a continuous gradation of large ecological extent within a small spatial extent.

This *continuous morphism*, as we may call it, as opposed to morphism of the usual discontinuous type, can be regarded as derived from the common phenomenon of broad ecogeographical differentiation by the spatial narrowing and compression of the ecological gradients concerned, until the allopatric populations become a single sympatric one. It is thus in a sense the converse of the secondary geographical polymorphism which may arise through the spatial extension of a morphic ratio-cline until one or both of its ends become monomorphic (p. 13).

We may expect to find many other cases where morphism becomes continuous and so grades over into high normal variability. Egg-number morphism in birds, when large clutches are involved, is one

case (p. 10). The high variability of the egg-markings of the Guillemot, *Uria aalge*, is perhaps another (p. 9 ; and see p. 52).

Very occasionally morphisms occur in which extrinsic advantage depends on the simultaneous presence of all the morphs concerned. Such morphisms may be called *synergic*.

One such example is the cryptic resemblance of various species of the hemipterous bug, *Flata* (*Phromnia*), to flower-spikes, which is effected by a dimorphism, one morph being green like the buds, the other being coloured like that of the flower of the species copied. This synergic resemblance has deceived human observers, and must be quite effective against natural predators (see Cott, 1940, p. 344, and Gregory, 1896).

The Ruff, *Machetes pugnax*, is unique in its extreme polymorphism of male epigamic (display) characters, no two males on a mating-ground being alike. The highly developed display-characters—ruff and " ear-tufts "—are of different colour and pattern from the general plumage, and often from each other. One function of display is to stimulate psychophysiological development of the reproductive and mating system, and the stimulation is enhanced when display is communal. Ford's (1945) suggestion that the striking variety of the Ruff's display adds further to the effect, is probably correct. However, the uniqueness of the phenomenon remains unexplained. If polymorphism of display characters is advantageous, why has it not been evolved by other avian species with communal display?

Bird " duets " in which male and female sing complementary parts of a single song, either simultaneously or successively, may also be regarded as synergic morphisms (Huxley, 1955).

3. PRESUMED ADAPTIVE MORPHISM : RATIO-CLINES

There are many cases of morphism to which no adaptive value can be as yet ascribed but where we may presume that the phanero-morphism is the outward and visible sign of an inward and cryptomorphic adaptive grace, as in *Colias*. This presumption is much strengthened whenever a ratio-cline exists, for this immediately shows that the morphic balance is being affected by environmental conditions and suggests what factors to explore.

Numerous cases are mentioned in the literature (see *e.g.* Mayr, 1941 ; Huxley, 1942 ; Dobzhansky, 1951 ; Ford, 1945*a*, 1953, 1955). A good example is provided by the Common Guillemot, *Uria aalge* (Southern, 1951 ; and see Fisher and Lockley, 1954, p. 48). This is dimorphic for a white " bridle " or " spectacle " marking round the eye (var. *ringvia*). In the normal unbridled morph the position of the bridle is marked by a slight groove. In the Atlantic, bridling shows an irregularly stepped cline of increasing frequency from under 0·5 per cent. in the south to over 50 per cent. in some northern areas. This straightforward picture was upset when it was found that the bridling frequency declined from over 50 per cent. in South Iceland

to a considerably lower figure in North Iceland. However, it was then pointed out that the climate of South Iceland is markedly Atlantic, while that of North Iceland is continental. Thus the shape of the ratio-cline suggests that the environmental factor favouring the bridled morph is not merely a function of north latitude, such as lower mean temperature or longer summer day-length, but a combination of this with a threshold value for humidity.

Uria aalge is interesting in another respect: though the range of the species extends both to the North Pacific and the North Atlantic, the bridled morph occurs only in the Atlantic (though in several populations classified as distinct subspecies). One must presume that the bridled mutation occurred only in the Atlantic, after the two main populations of the species had become separated.

Southern has further shown that the allele for bridling also determines slight differences in the structure of the skull and the shape of the tail-feathers, so that here again a non-selective " visible " character is correlated with more deep-seated changes. A repetition of the census after ten years showed significant changes in bridling frequency in a number of localities, some being increases and others decreases. So far it is impossible to correlate these with the known amelioration of climate during the period, but further repetitions should eventually permit definite conclusions.

In foxes, the Arctic Fox, *Alopex lagopus*, shows dimorphism in winter (blue versus white), and there is a N.-S. ratio-cline. The Red Fox, *Vulpes fulva*, is trimorphic, the heterozygote (" cross ") being distinguishable from both homozygotes, the common red and the rare " silver " (black). Here too there is a ratio-cline, silver (and of course also cross) increasing in frequency to the N. The delicacy of the selective balance is also shown by the fact that the frequency of silver changes cyclically in relation to the animal's regular ten-year cycle of abundance. All races of *V. fulva* show this morphism, but it appears to have developed independently in the Old and New Worlds, the black allele of the true silver fox occurring only in Canada, while a different black allele occurs in Alaska and Eurasia (see Huxley, 1942; Voipio, 1950).

Wild white clover, *Trifolium repens*, shows an interesting ratio-cline (Daday, 1954) in the distribution of HCN, causing bloat in ruminants. The production of HCN depends on two allele-pairs, the dominant members of which are concerned with the presence respectively of a cyanogenetic glucoside, and of an enzyme hydrolysing the glucoside. Both allele-pairs show ratio-clines dependent on winter temperature, a decrease of 10 per cent. in January mean temperature resulting in a reduction of 4·23 per cent. in frequency of the dominant glucoside allele, and of 3·16 per cent. in that of the dominant enzyme allele. The glucoside allele thus fades out slightly earlier with decrease of January temperature, but the behaviour of the two genes is sufficiently similar to give a straightforward phenocline as regards HCN-production.

A rather similar case is that of the Castor Bean, *Ricinus communis* (Harland, 1947), in Peru, where it occurs as a widespread introduced weed as well as in cultivation. Here a morphism exists involving the presence or absence of waxy bloom on the stems. Bloom is due to a dominant allele, *B*; this increases in frequency with altitude, which connotes increase of sunlight and decrease of fog, from 0·15 per cent. at sea-level to 100 per cent. at 7700 feet. The selective disadvantage of *B* plants at low altitudes is due to their much-reduced ability to fruit in sunless and foggy areas.

Doubtless numerous other character-clines, such as are subsumed under the various biological rules (see Rensch, 1954, ch. 3), will prove to have a similar genetic basis, though in many of them this is likely to be multifactorial, leading to continuous morphism.

In the Deadly Nightshade, *Atropa belladonna*, Dr J. H. Burnett tells me that unpublished work indicates the existence of a yellowish-green-flowered recessive morph which is found wild in this country only in sheltered habitats, and has less hardy seedlings than the normal purples. There is a ratio-cline, the yellow-green morph increasing in frequency eastwards; but other characters also gradually appear, so that the easternmost group of the *Atropa* population has been distinguished as a separate species, *A. lutescens*.

The full analysis of this case will be of great interest, as would that of the hairy- versus smooth-stemmed morphs of the Foxglove, *Digitalis purpurea* (Saunders, 1918), hairiness being dependent on a recessive allele which is at a slight viability disadvantage against the heterozygote. Both types are widely distributed over England, but the ratio varies much. Some other species of *Digitalis* are monomorphic smooth, others monomorphic hairy.

In the marine isopod *Sphaeroma* (Bocquet, Levi and Teissier, 1951), there are five main colouration phenomorphs, based on a system of alleles (or closely-linked genes); some show ratio-clines; (see p. 52).

The colouration tetramorphism of the females in the Lycaenid butterfly, *Neozephyrus taxila*, depends on a system of three alleles or semi-alleles, the two dominants suffering an intrinsic disadvantage in general fitness. The recessive appears to enjoy an extrinsic (cryptic) advantage. The morph-ratios vary geographically (Komai, 1953).

The Pied Flycatcher, *Muscicapa hypoleuca*, is broadly dimorphic in the male (black or brown) for its plumage-pigmentation (Witherby *et al.*, 1938-51; Drost, 1936). The difference appears to depend on the different rates of action of pigmentary rate-genes, as in the similar morphisms of some male Geospizidae (Lack, 1947); and there are geographical ratio-clines. For numerous other examples of morphism in birds, see Huxley, 1955.

The common British spider *Theridion ovatum* (*redimitum*), is sharply trimorphic—white, red, or red-striped white. This probably depends on a single allele-pair, the heterozygous red-striped enjoying some advantage, while homozygous reds suffer some intrinsic disadvantage. Morph-ratio varies markedly: thus whites range from 50·8 to 87·1,

striped from 9·7 to 44·4, and reds from 0 to 11·8. Further, there is a ratio-cline, with white frequency increasing to the W. A puzzling result was yielded by two large collections made in one day at Brockenhurst: the afternoon collection gave abnormally high red and abnormally low striped frequencies. This case would repay further study (Bristowe, 1931).

In contrast, the anticryptic colour-dimorphism of *Misumena vatias*, which adapts the spider to the flowers on which it sits, is non-genetic, occurring physiologically in about 48 hours (Bristowe, 1941, *2*, 435; Gabritchevsky, 1927).

The shell-colour (and -form) morphism of the Dogwhelk, *Purpura* (*Nucella*) *lapillus*, has several peculiar features (Moore, 1936). It has six phenomorphs for ground-colour and banding, and one for imbricated shell. The morph-ratios show marked but rather erratic geographical differences. Some of them are adaptively correlated with habitat; thus the more delicate imbricate morph is almost confined to habitats below the reach of wave-action, as one would expect. But high yellow frequency shows an unexplained correlation with moderate wave-exposure, the frequency being low both in sheltered and exposed situations. Finally, the black-brown, mauve-pink, and white morphs are dependent on diet as well as on genetic factors, the two former only developing on a diet of mussels, *Mytilus edulis*, the last-named only on an acorn-barnacle diet. If black or mauve specimens are transferred to an acorn-barnacle diet, the new shell will be white. This remarkable case demands investigation by modern methods.

Limpets (*Patella*) are much less variable (Evans, 1947); but there is some morphism of foot-colour in some species, and of colour of shell interior; this latter may show geographical variation in morph-ratio.

Some ratio-clines propound a puzzle rather than a clue. For instance, in the dimorphic Fulmar, *Fulmarus glacialis*, the frequency of the dark morph in the Atlantic is very low in the south of the breeding range, very high in the north. But in the Pacific subspecies the relation is reversed! (J. Fisher, 1952).

Again, though all the smaller Skuas of the genus *Stercorarius* are dimorphic, with dark and light plumage-morphs, and the Arctic Skua, *S. parasiticus*, shows a ratio-cline with increased dark-frequency to the north, largely correlated with increasing humidity (Southern, 1943), in *S. longicaudus* the dark morph is exceedingly rare, and there is apparently no cline in *S. pomarinus* (Witherby *et al.*, 1938-51; Southern, 1944).

Such an absence of any ratio-cline is itself a puzzle, as one would *a priori* expect that environmental conditions would normally affect the delicate selective balance involved. However, the Gouldian Finch, *Poephila gouldiae*, in Australia is dimorphic, roughly 80 per cent. of the birds having a black mask and 20 per cent. a red one, with the gene for red a sex-linked dominant. But the frequency is stated to show no clinal alteration over all of its large range (Southern, 1945). A similar absence of true ratio-clines (as opposed to changes in

morph-ratio due to gene-diffusion after crossing) occurs in human blood-group morphisms (see later). In such cases, possibly the intrinsic selection-pressures as regards various components of general fitness are so closely balanced that the balance is unaffected by changes in extrinsic (environmental) conditions. Differences in ratio between different groups (*e.g.* in ABO ratios in human ethnic groups) could then be due either to slight allelic differences in the main morphic genes, or to different combinations of modifiers affecting their intrinsic selective values (see p. 34).

Sometimes the ratio-distribution of morphs is not regularly clinal, but yet can be correlated with some ecological factor. Thus the dimorphism of forceps-length in the male Earwig, *Forficula auricularis*, appears to be correlated with conditions favouring abundance and large size (see Huxley, 1927 ; and pp. 28, 52).

The morph-ratios of blue- versus white-flowered plants in *Linanthus parryae* show a curious haphazard distribution (Epling and Dobzhansky, 1942 ; Dobzhansky, 1951, p. 168). Dobzhansky considers that some of this is due to non-selective drift, though it must depend partly on some unknown selective factor (Wright, 1943).

4. ORIGINS AND EVOLUTION OF MORPHIC SYSTEMS

Genic balance-systems may originate from single mutant alleles which happen to have both favourable and deleterious effects : such genes may be called *ambivalent*. Further, they may show either *full ambivalence*, both effects being then exerted in both heterozygous and homozygous dosage, or *dosage ambivalence*, in which the deleterious effect is exerted only (or much more strongly) by the gene when in double dose.

In fully ambivalent genes, if the partial dominance of the favourable effect happens to be greater than that of the deleterious one, a condition of slight double-dose disadvantage will arise and will favour the heterozygote, thus tending to maintain the allele in permanent balance in the population. Furthermore, as Dr P. M. Sheppard has pointed out to me, selection will automatically operate to increase the dominance of the favourable effect and the recessivity of the deleterious one, thus further strengthening the stability system.

A good example of such ambivalent genes in morphic balance in a species is provided by the moth *Ephestia kühniella* (Caspari, 1950). This species is morphic for testis-colour, a considerable proportion being *rt.rt* in constitution, with red testes, as against *Rt*, with brown testes. Testis-colour can obviously have no extrinsic adaptive significance, and the dominance of *Rt* in this respect is presumably, as Caspari suggests, a genetic accident. However, the morphic alleles do exert selective effects. Thus *rt.rt* individuals develop slightly more slowly than either *Rt.rt* or *Rt.Rt*, but enjoy a large pre-emergence viability advantage over *Rt.Rt* homozygotes and a small one over all *Rt* individuals combined (though a slight disadvantage as against the

heterozygotes). In mating behaviour (success in effecting copulation), on the other hand, *rt.rt* homozygotes are at a marked disadvantage against heterozygotes, and at a slight disadvantage against *Rt.Rt* homozygotes. *rt* is partially dominant for its favourable effect on viability, but largely recessive for its unfavourable effect on mating behaviour: the result is heterozygote advantage, with balanced morphism. Gustafsson (1953) has analysed several similar cases of "monofactorial heterosis" in barley. (See Haldane, 1954, p. 64, for biochemical implications.)

As an unexpected selective correlate of a mutant character, increased hairiness in cotton confers a (mechanical) resistance to attack by Jassid bugs such as *Empoasca* and has resulted in hairier races in various areas (Hutchinson, 1951). If the hairiness gene had shown double-dose disadvantage, a morphism would have arisen.

Teissier (1953) has shown how mutant genes in *Drosophila* may spread in certain experimental conditions. A. H. Jackson (in press) has obtained somewhat similar results with white-eyed *Drosophila* in darkness, and Waddington *et al.* (1954) with the temperature and humidity preferences of different laboratory stocks: but their suggestion that this might lead to "a relatively stable polymorphism" would only hold if inversions or double-dose disadvantages were involved.

Another type of gene capable of originating a genic morphism is one in which the heterozygote enjoys the advantage of greater flexibility because its effect is not that of one (dominant) allele alone, nor merely intermediate, but that of both allele-partners simultaneously (Smith and Smith, 1954). When this double effect is of a biochemical nature, the resultant biochemical diversity may confer marked advantage on the heterozygote, as in sickle-cell anæmia (Pauling *et al.*, 1949; Haldane, 1954, p. 121). Such genes may perhaps be called diversifiers; see p. 39.

Once a single-gene morphism is established, it may evolve in a number of ways. The double-dose disadvantage may be increased, and the stability mechanism accordingly strengthened, by the incorporation into the system of other mutants with double-dose disadvantage. Once a strong double-dose disadvantage is set up, whether by the above means or through the recessive lethality of the original ambivalent gene, further recessive lethals may become incorporated in the system, by mutations at loci close to the original morphic locus (p. 7). The selective disadvantage of dominant morphic homozygotes in the grouse-locust, *Paratettix*, is as high as 7 to 14 per cent. (Fisher, 1939).

Furthermore, all morphic alleles in the same chromosome which show double-dose disadvantage will tend to become more closely linked, since crossing-over will give selectively more unfavourable combinations. This was pointed out by R. A. Fisher nearly a quarter of a century ago (1930, p. 102 ff; and see p. 7).

The tendency to closer linkage will operate when, as with many dominant morphic genes in grouse-locusts, each shows double-dose disadvantage: it will do so with especial force when different dominant genes interact so that when present together the sum of their double-dose disadvantages is increased; or in the curious cases, also found in grouse-locusts, when co-presence converts two separate single-dose selective advantages into a joint selective disadvantage.

Since selective interaction is more likely to occur with genes affecting the same type of character, close linkage is frequently found between many members of a single morphic system.

Fisher (op. cit., p. 110) further showed that closer linkage will be favoured between loci having similar quantitative effects on metrical characters. Accordingly, if any such gene exists close to a morphic gene, other metrical genes in the same chromosome will tend to become closely linked with the morphic gene-system. Sheppard has pointed out that translocations containing genes of these types will also be favoured.

As a result of these tendencies, many species come to possess morphic systems of closely-linked genes. Thus in *Paratettix texanus* 24 of 25 morphic genes behave as semi-alleles: in *Apotettix* almost all the many genes concerned with morphic patterns are in one chromosome, and mostly in a few extremely closely linked groups (Nabours and Stebbins, 1950). Similar morphic gene-groups are known in other grouse-locusts (Fisher, 1939), and in grasshoppers (Rubtzov, 1935), in mammals for blood-group characters, in snails for shell-characters, in Cyprinodont fish for colours and patterns, and in various other organisms; and may show semi-allelism (Komai, 1950). In the grouse-locust *Acridium* (Nabours *et al.*, 1933) the 12 morphic genes are all in one chromosome, but not closely linked.

A quite different type of origin for morphism is provided by inversions, as in *Drosophila*, and this may lead on to euheterosis (see p. 11).

The fact that a morphic chromosegment contains a large number of genes, mostly producing different effects, and that the two members of the system probably differ *ab initio* in a large number of loci, makes it likely that they will show larger original differences in selective values, both quantitatively and qualitatively, than will genic systems. This will also make it possible for the morphic chromosegments to show secondary adaptive evolution as wholes, by means of the incorporation of suitable mutants (supergene systems), so that each comprises both a switch-mechanism and the adaptive gene-system dependent on the switch and canalising its effects, and also half of the co-adaptive euheterosis mechanism (p. 12). With other types of origin, the switch-mechanism and the dependent canalisation-mechanism may be and usually are localised in different regions of the gene-complex. However, this need not always be so, as in *Primula* (p. 20).

In most types of genic morphism, secondary adaptive evolution will also tend to occur. Thus in polymorphic mimetic butterflies there is the mimetic adaptation of the original morphs through the evolution of switch-dependent canalisation mechanisms. These mimetic adaptations must be closely adjusted by selection (p. 6).

The cryptic adaptations of colour and pattern in the morphism of *Cepaea* appear also to be due to the selective improvement of crude " pre-adaptive " resemblances exhibited by some of the morphs (p. 9).

In general, we must expect that selection will operate on any primary intrinsic morphism in such a way that it will gradually show secondary extrinsically adaptive modifications in some or all of its morphs ; the more ancient the morphism (as shown by its presence in several related species or genera) the more likely it is to show secondary adaptations. The detection of such secondary adaptation, however, is often difficult. Selection may also be theoretically expected to act so as to reduce the deleterious effects of ambivalent morphic alleles, by buffering them with modifiers. I do not know of any case in which this has been conclusively proved, but it may possibly occur in the fish *Platypoecilus* (p. 18) and in human sickle-cell anæmia (p. 39).

The original discontinuity between the morphs in all examples hitherto mentioned is genetic, provided by mutation of some sort. But in other cases it may be developmental, provided by the existence of large meristic units or of distinct growth-stages and moult-instars. I have already discussed avian clutch-size as an example of the former ; other examples of meristic discontinuity as a basis for morphism doubtless exist, *e.g.* in vertebra-number in fish.

Instar-discontinuity is probably the basis for the dimorphism in forceps-size of male earwigs, *Forficula auricularia* (Huxley, 1927). Some sort of limitation of growth presumably underlies the continuous polymorphic variation of size and mandibles in various beetles, and of size and head of neuters in some species of ants, as well as the sharp size-dimorphism seen in others (Huxley, 1932). But much experimental analysis of such cases is still required.

The existence of alternative " mutational channels " (Mayr, 1942, p. 74), or, as they might be called, *canalisations of variance*, may enlarge the discontinuities produced by mutation. A similar large discontinuity may be provided by the existence of alternative ontogenetic processes or *canalisations of development*, such as Waddington and other experimental embryologists are now busy investigating.

However a morphism may originate, and whether its original basis is intrinsic, concerned with general fitness, or extrinsic, concerned with adaptation to external conditions, it will tend to evolve in the direction of greater stability and higher adaptive value.

5. UNEXPLORED MORPHISMS AND PROBLEMS FOR FURTHER RESEARCH

Many cases of morphism I have found in the literature have either been wholly unexplored or demand further investigation.

The well-known female dimorphism of the Currant Moth, *Abraxas grossulariata*, is unique in that the " mutant " *lacticolor* (*dohrnii*) produces a virus or virus-like body which reduces viability. The normal morph can be infected with this, but cannot produce it (Smith, 1952). Darlington suggests that the virus is a detached plasmagene to permit the permanence of the morphism. Lacticolor is not in morphic balance, but only a rare mutant. Nevertheless the case is worth citing as an example of an unexpected " correlated character " of selective import.

The frequency of melanic Leopards (*Felis pardus*) varies much. Though black Servals are not uncommon in E. Africa, black Leopards are rare except at high altitudes (Foran, 1952). They are very rare in India, but increase in frequency eastwards (T. G. Longstaff, *in verbis*). The colour-morphism of the American Black Bear, *Euarctos americanus*, differs in different subspecies. *E. a. americanus* is typically black with brown and cinnamon morphs ; *E. a. emmonsu* is black or more rarely slate-grey ; *E. a. kermodei* is typically white, but with a dappled reddish-yellow morph. In the Brown and Grizzly Bears, *Ursus arctos*, one montane subspecies (*U. a. isabellinus*) is markedly polymorphic, in contrast to the numerous other races.

Among birds, a unique case of morphism in habits was described by H. J. Frith at the International Ornithological Congress in 1954 for the Megapodidae (Mound-builders). Several species of *Megapodius* and *Leipoa* are di- or poly-morphic in their mound-building habits. Thus in a single population of one *Megapodius* species there are birds which dig holes in sand, others which build large mounds, either solely of organic matter or of sand enclosing organic matter, and still others which utilise volcanic heat. Geographical polymorphism also occurs.

What appears to be a unique case of vocal morphism has recently been described in the Tawny Owl, *Strix aluco*, by Muir (1954). Young birds, even from the same brood, give one or other of two quite distinct calls.

Many reptiles and amphibians are morphic, including all species of snakes, lizards, *Anura*, and *Urodela* (Smith, 1951) found in Britain, though the sand lizard has a melanic morph only on the Continent. The remarkable blue-spotted morph (var. *colchica*) of the Slow-worm, *Anguis fragilis*, is found only in adult males : it shows an increasing frequency to the southward. It is interesting to note that other species of Anguidae, e.g. *Ophisaurus gracilis* and *O. harti*, are monomorphic in both sexes for this character. In *Anura* the genetic colour-morphism is complicated by the animals' capacity for physiological colour-change. In some areas, *e.g.* Scotland, the red morph of *Rana temporaria* is unusually frequent.

In the Alpine Newt, *Triturus alpestris*, in some high Alpine lakes " a breed has evolved in which [total] neoteny is common " (M.

Smith, op. cit.). This morphism may be an evolutionary development from the partial neoteny seen in the British Smooth Newt, *T. vulgaris*, where a certain proportion of individuals in steep-sided artificial ponds find emergence too difficult, and remain unmetamorphosed in the water (but do not mature sexually). A high proportion of such forms also show partial albinism (*cf.* Main's frogs, p. 17). Similar metamorphic morphism seems to occur in Axolotls (see Bateson, 1913, p. 231).

The American King-snake, *Lampropeltis zonata*, is highly variable both between and within geographical populations (Zweifel, 1952). Some of this variance is apparently morphic, but some probably due to recombination after secondary hybridisation of subspecies.

Molluscs provide an almost inexhaustible supply of morphisms; such striking cases as the four British Periwinkles, *Littorina* (Yonge, 1949). E. Mayr (*in litt.*) tells me that in *Littorina obtusata* he has found a correlation between morph-frequency and colour of substrate, as in *Cepaea* (p. 9). Among the sea-slugs, there is *Archidoris* (*Doris*) *britannica*, where some morphs appear to be procryptic, the trimorphic *Goniodoris nodosa* (Yonge, *op. cit.*), and many cases in Alder and Hancock (1845). Among Eolids, though morphism occurs in some species, like *Eolis papillosa*, it is rarer. This is presumably because many of them have aposematic colouration advertising the unpleasant properties of their stored nematocysts, so that it is of biological advantage to be monomorphic in the pattern to be learnt by their enemies.

Prof. R. Seshaiya of Annamalai University has given me a collection of shells of the marine gastropod *Umbonium vestranum* from south of Madras, which makes it clear that the polymorphism of this genus is so extreme as to rank with that of *Apotettix* or *Lebistes*.

Land-snails and slugs appear to be exceptionally polymorphic: *e.g.* Bateson (1913) cites *Helix heripennis* with 27 morphs, and *Bulimus detritus* with 18 morphs; and see Komai (1954) on *Bradybaena*.

Among Lamellibranchs, Sowerby (1847-82), Step (1901), and Yonge (*op. cit.*) record a number of morphisms affecting either shell-colour or body-colour or both, *e.g.* in common British genera like *Tellina*, *Tapes*, *Donax* and *Pinna*. Extreme polymorphism is shown by various species of *Pecten* (*sensu lato*) such as *P. proteus* and *P. tigrinum* (see Sowerby's pls. XII to XXIV, and Step, p. 85). *Tridacna elongata* and *T. marina* of the Great Barrier Reef, show a remarkable mantle-colour morphism, while others are wholly or nearly monomorphic (Yonge, 1930). Bateson (1913, p. 130) suggests that *Anodonta* is morphic as well as highly variable.

Among Crustacea, various isopods show morphism in colour and pattern, *e.g.* the common woodlice, *Armadillidium vulgare* and *Porcellio scaber*. The latter is tetramorphic, including a non-patterned morph which is presumably the universal recessive. Morph-ratio differs in different habitats (Meinertz, 1943). Howard (1953) has begun the genetic analysis of the morphism of *Armadillidium*. In the Turkish

woodlouse, *Cyclodiscus convexus* (de Lettin, 1952) there are three female reproductive morphs, producing both sexes, almost only females, and almost only males respectively. Two morphic allele-pairs seem to be involved. For *Sphaeroma*, see pp. 25, 52.

In Copepoda, Bocquet (1951) has recently analysed the pattern-morphism of the marine *Tisbe reticulata*. There are seven main phenomorphic patterns in females, three in males. They depend mainly on a genetic system of several main dominant alleles (or closely linked genes) and a universal recessive, but with the addition of two recessive genes, and other genes producing a " secondary polychromatism " affecting small groups of pigment-cells only.

The Common Shore-crab, *Carcinus maenas*, is remarkable in showing a bewildering polymorphism of colour and pattern in juveniles up to about an inch in carapace-breadth, while adults are much less variable (personal observation). Possibly the variety of patterns in juveniles is adaptive as in *Cepaea* (p. 9), by conferring cryptic resemblance in a wider range of habitats. An extensive study of the morphism of this abundant species would be of great interest.

Echinoderms reveal what is to me a surprising number of colour- and pattern-morphisms, including that of the bêche-de-mer, that much-prized holothurian (Yonge, 1930). Among British species we have the dimorphic Spring Starfish, *Marthasterias glacialis*, the polymorphic Scarlet Starfish, *Henricia sanguinolenta*, and the trimorphic Sun-star, *Solaster pappus*, in the Asteroids ; and in the Ophiuroids the highly polymorphic Common Brittle-star, *Ophiothrix fragilis* and its relative *Ophiopholis aculeata* (Yonge, 1949). Frequency determination of the morphs of *Ophiothrix* in different regions could now be carried out on an extensive scale by means of under-water photography, and would be of great interest.

Among Compound Ascidians the abundant Golden-Stars sea-squirt, *Botryllus schlosseri*, is tetramorphic in colour as regards its gelatinous matrix, and dimorphic as regards its individual zooids ; and among Sponges the common British species, *Halichondria panicea* and *Hymeniacidion sanguinea*, are trimorphic in colour (Yonge, 1949).

A thorough ecological, experimental, and perhaps even genetic study of morphism in sea-anemones should be rewarding.

Ford (1955) has a valuable discussion of many morphisms in moths, including the now classical *Panaxia dominula* (Sheppard, 1951).

What may possibly be a case of morphism in the Leptomedusan *Phialidium* is mentioned by Burkenroad (1931). In certain regions, a form occasionally appears in high frequency which differs from the type chiefly (but not wholly) in possessing five instead of four radial canals. This may be a meristic morph, but there are complicating details suggesting the alternative explanation that it is a distinct species or subspecies.

Among flowering plants, inspection will often reveal the existence of colour- and pattern-morphism. Thus from my own casual observations I can say that the Field Scabious, *Krautia* (*Scabiosa*) *arvensis*,

may show marked variation interpretable on the basis of two pairs of morphic alleles, one for general flower-colour, the other for paler marginal florets; that a Swiss population of Balm (*Melissa*) clearly contained at least three gene-pairs in equilibrium; that an English population of Hemp Agrimony, *Eupatoria cannabina*, was dimorphic for depth of colour both of flowers and stems; and that wild stands of the Fritillary, *Fritillaria meleagris*, always vary in intensity of flower-colour and often contain a white morph in moderate frequency.

Turrill (1948) cites a number of other cases. Among these I may mention the common Comfrey, *Symphytum officinale*, which is polymorphic in flower-colour (and I believe in density of green in leaves), while other species of the genus are monomorphic; the Musk Mallow, *Malva moschata*, and the Yarrow, *Achillea millefolium*, both dimorphic for white and coloured flowers, but the latter being unusual in that the coloured morph is the less frequent; and the Cuckoo-pint, *Arum maculatum*, which is not only strikingly dimorphic for spadix-colour, but also polymorphic for leaf-spotting. Some apparent cases of morphism may be wholly or partly due to recombinational variance after hybridisation, *e.g.* various orchids (Heslop Harrison, 1951; and Bateson, 1913, p. 125 for *Ophrys apifera* × *aranifera* × *muscifera* in some areas) and Comfrey; and others to ploidy or apomictic variation (*e.g. Ranunculus acris* and *R. ficaria*).

Summerhayes (1951) also lists numerous examples of morphism in British orchids, including the curious case of the rare "wasp" morph of the Bee Orchis, *Ophrys apifera*, which mimics a wasp's abdomen instead of a bee's: see also Heslop Harrison's interesting studies (1951, 1953).

The gum *Eucalyptus citriodora* shows biochemical morphism. A wild population in a restricted area contained a number of morphs differing in the chemical nature of their essential oils. Similar biochemical morphisms have been found in other eucalypts and in various *Myrtaceae* and *Rutaceae* (Penfold and Willis, 1953). It would clearly be of great interest to see whether ratio-clines exist in such forms, and how chemical peculiarities are correlated with environmental conditions.

Blackman and Rutter (1950) claim that the Bluebell, *Scilla non-scripta*, exists in strains of different bulb-weight. They have not, however, analysed the share of environment and genetic constitution in determining bulb-weight, so that it is dubious whether this involves true morphism. Schütte (1949) found that *Romulea bulbocodioides* near Cape Town was dimorphic (yellow versus white) in flower-colour, and that the morph-ratio varied markedly in different localities, with considerable areas monomorphic for one or other morph. His genetical deductions, however, appear to be without foundation.

A perusal of the recent British flora (Clapham, Tutin and Warburg, 1952) reveals large numbers of definite or probable morphisms. Here I can only mention a few specially interesting examples.

The Wild Carrot, *Daucus carota*, has a very peculiar and quite

frequent morph in which the central flower of the umbel is red or purple instead of white like the rest. Some species of Milkwort (*Polygala*) are markedly dimorphic in flower-colour, others trimorphic. The subgenus *Viola* contains some species in which a white or pale flower-colour morph is absent, others in which it is present but rare, and one (*V. odorata*) in which it is about as abundant as the coloured morph. In this last species, the white morph has become more abundant in recent decades in certain areas (Turrill, 1948, p. 133; and see Walters, 1944).

In the subgenus *Melanium* (pansies), the colour-polymorphism is notorious, and greater than in the nominate subgenus. *Veronica*, many umbellifers, *Dianthus*, *Silene*, *Convolvulus*, *Calystegia*, *Datura*, *Verbascum*, *Limosella*, *Rhinanthus*, *Melampyrum*, *Euphrasia* show colour-morphism in many species (in the last-named genus inter-specific hybridisation may be responsible for some of the variance). Many genera of *Boraginaceae* show different degrees of morphism in different species. In some of them the phenomenon is complicated by the fact that flower-colour may change from red to blue or purple during development: this combination of genetic with developmental morphism is paralleled in birds (p. 23). The Bladder Campion, *Silene cucubalus*, is dimorphic in respect of hairy or glabrous stems. Mr Marsden Jones tells me that the Meadow Saxifrage, *S. granulata*, includes populations which are highly polymorphic for petal-size and shape (and *cf.* Digitalis, p. 23).

Field botanists will know of many other examples, and geneticists will doubtless find numerous fascinating problems for investigation in common plants.

This list of morphisms could be enormously enlarged; but I have said enough to show what a wealth of interesting problems await analysis in this field.

6. MORPHISM IN MAN

Morphism in man deserves a special section, partly because it sheds light on human genetics and its medical, social, and ethnic applications, and partly because certain important types of morphism were first discovered and are most easily studied in our own species.

Most human polymorphism, in the general sense of high variance, is either geographical or due to hybridisation between previously isolated geographical populations. True morphism, however, is also widespread. One universal morphism is that of the blood-groups. Admirable detailed accounts of the subject are available, such as those by Race and Sanger (1950) and Mourant (1954); see also Boyd (1950); here I shall confine myself to points of general biological interest.

To begin with, some at least of the blood-group morphisms are very ancient, such as the ABO system, which exists in a somewhat

different and not yet fully analysed form in anthropoid apes as well as in Homo (see Boyd, 1950, p. 334; Mourant, 1954). Its origin must accordingly date back at least to the Miocene.

As already mentioned, other higher vertebrates also show analogous blood-morphisms (cattle, Stormont, 1952; ducks, McGibbon, 1945; doves, Irwin, 1953; fowls, Briles *et al.*, 1953). In doves the antigenic characters help in determining taxonomic relationships. It would be of great interest to extend such studies to other species, especially to the wild relatives of domestic forms. See also p. 52.

In general, blood-group morphisms depend on systems of allelic or more probably semi-allelic (very closely linked) genes. In fowls there is a system of four semi-alleles. In doves (*Streptopelia spp.*) there are up to nine antigenic morphs in one system. In cattle there are four such systems, two dimorphic, one with over twenty and one with over eighty morphs. This last group possibly depends on a system of up to twenty semi-alleles, and if so is one of the largest morphic systems known.

In man, there are already nine systems known, or ten if we include the secretor system, which is linked with the Lewis blood-group, and determines whether the A, B and other blood-group substances are secretable into the saliva and other secretions. Details can be found in the works already cited (and see Sheppard, 1953; Roberts, 1954*a*; Race *et al.*, 1954).

Different human ethnic groups show different morph-ratios and some may lack certain morphic genes of a particular blood-group system. It would be of great interest to discover if animal species show similar intra-specific variation in blood-group morph-ratios.*

It appears that these ethnic (" subspecific ") differences in morph-ratio are extremely resistant to environmental influences, and remain stable even after migration to quite new habitats, thus incidentally providing one of the best indices of evolutionary relationship between different human races. A similar but lesser stability characterises other blood-morphisms such as sickling (see later), as demonstrated by Lehmann, 1954. This important field of comparative human genetics is being actively prosecuted, but there are still great gaps in our knowledge (Mourant, 1954, p. 194).

Man's blood-groups thus constitute outstanding examples of an intrinsically stable morphic balance-mechanism; and the question at once arises how this stability is achieved for the various equilibrium-points actually found. However, this is easier asked than answered. Theoretically, we must suppose that the intrinsic (general fitness) selective advantages and disadvantages of the various morphic genes in each system are so nicely balanced that a highly self-regulating equilibrium is reached—a conclusion borne out by the recent work of Allan (see later). Differences in ratio between ethnic groups would

* The present terminology of the blood-group genes is confusing and genetically barbarous. It is good news that E. B. Ford (1955; A uniform notation for the blood-groups; *Heredity*, (this issue) is proposing a satisfactory and universally applicable system which is in line with current genetic principles and practice.

then presumably be due mainly to differences in modifiers in the rest of the gene-complex affecting the expression of the various main morphic genes and so altering their effective advantages and disadvantages, as suggested by Ford (1945), though it is theoretically possible that some of it may be due to mutation of the morphic genes themselves. In any case, it seems clear that the suggestion of Wright, that the ratios originated through drift, and then remained stable, is not likely to be true.

The continual existence of a number of alternative morphic genes in equilibrium must involve a selective balance, as demonstrated by R. A. Fisher a quarter of a century ago. In spite of this theoretical necessity, almost all medical authorities have until recently maintained that the blood-group morphisms had no selective implications, and this view has been supported by leading geneticists (*e.g.* Wright, 1940 ; Dobzhansky, 1951, p. 157).

Recently, however, the position has changed with almost dramatic suddenness. In the first place, the Rh (rhesus) system obviously has selective implications because of the deaths from erythroblastosis fatalis due to iso-immunisation in certain genetic combinations of mother and offspring (Haldane, 1942). This would operate to reduce the number of viable heterozygotes between Rh-positive and Rh-negative, *e.g.* in such combinations as *Dd*, and so to reduce the frequency of the rarer gene—here *d*, or Rhesus-negative—in the population. Glass (1950) has shown that this tendency, is, in populations which limit family size, such as U.S. whites, compensated for by the opposite tendency to make up for deaths of offspring by adding more children to the family. It does not operate in U.S. negroes, where family limitation is little practised. This, however, cannot well be the whole story. Rh-negative genes presumably possess some as yet undiscovered intrinsic advantage which permitted them to reach morphic balance in the populations in which they originally arose. The situation is further complicated by ethnic crossing in a way which does not seem to apply to the other blood-groups.

With these too, however, evidence of differential selective value has recently been discovered. Many years ago, R. A. Fisher suggested that a determination of the morphic ratios of the blood-groups in different age-groups within a single population would probably reveal some differential viability. This challenge has now been taken up, and interesting though puzzling results have been obtained, not only on the differential viability of the ABO morphs, but also on their differential fertility (see Allan, 1953, 1954 ; Anon., 1954*a*). Briefly, the position is as follows : differential viability is marked, but is opposite in the two sexes. Vuori found in a large sample of Finnish children and adolescents aged eight to eighteen years that the $\frac{B}{A+O}$ ratio shows a progressive fall in males, from 0·27 to 0·18, but a progressive rise in females from 0·18 to 0·35. In both sexes there is a brief reversal of the trend in the thirteenth year at the beginning of

adolescence. The B girls developed more slowly, their average age at first menstruation being 14·7 years against 14·3 for A and O girls. The abortion rate for B mothers was nearly double that for (A+O) mothers.

Allan (1954) has independently indicated the existence of a similar state of affairs in the British population, the order of increasing fitness (viability) being O→A→B for females, B→A→O for males. He further found (Allan, 1953), on re-analysing Waterhouse and Hogben's data, that there is also an order of fertility as between the morphs, but as far as B is concerned, this is in the reverse sense from that for fitness. The order of increasing fertility in females is B→AB→O→A, and the opposite in males. We may express these curious facts in a diagram, in which the arrows point in the direction of higher fertility or viability.

Group	Viability		Fertility	
	♂	♀	♂	♀
O+A	↑	↓	↓	↑
B				

As regards B and (O+A) the differential, selective values are opposite in either sex for viability and for fertility, and are opposite in the two sexes in both cases.

This symmetry is not perfect when O and A are considered separately. The diagram then runs as follows :—

Group	Viability		Group	Fertility	
	♂	♀		♂	♀
O	↑	↓	A	↓	↑
A			O		
B			B		

Not all of these findings are fully significant statistically, but they are extremely suggestive and should be followed up on a large scale.

Meanwhile, other evidence of differential viability has been obtained. Johnstone (1954) has shown a significant difference of ♂ sex-ratio between the offspring of B mothers and non-B fathers, and the offspring of B fathers and non-B mothers—55·4±2·9 as against 47·5±2·7. Whether this is due to differential fertilisation by X- and Y-bearing sperms or to differential mortality of male conceptions *in utero*, we do not yet know. The latter would *a priori* be more likely,

but is difficult to reconcile with the higher fertility of B mothers as established by Allan. In any event, the effects of the two differentials approximately cancel out, so that there is no progressive alteration, either of sex-ratios or of B-frequency, in the population.

Johnstone failed to confirm the results of Sanghvi (1951), who claimed that the ♂ sex-ratio of O offspring of O mothers was higher than that of A offspring of A mothers (60·5 as against 49·8); possibly this may be due to the fact that Johnstone's sample was British, from London, Sanghvi's Indian, from Bombay. Sanghvi also cites data from New York which show the same trend (56·3 as against 45·7).

For toxæmia of pregnancy, O mothers are at a relative disadvantage, toxæmic patients being 53·6 per cent. O, as against 45·5 per cent. in controls; A and B mothers on the other hand are at a relative advantage, the incidence of A and B and AB in toxæmic patients being markedly lower than in the general population (Pike and Dickins, 1954).

General viability, of course, includes components relative to susceptibility to various specific diseases; and here our knowledge of the differential selective values of the ABO morphs has been much enlarged. Thus Struthers (1951) has shown that a significantly larger proportion of A than of O babies die of broncho-pneumonia during the first two years of life. Aird and Bentall (1953) have shown a similar result for incidence of stomach cancer, a higher percentage of A's being found in cancer patients than in the general population, a lower percentage of O's. The figures are as follows (per cent.):

A			O		
Stomach Cancer		Control	Stomach Cancer		Control
44·8	>	39·8	44·5	<	48·6

Later work shows that B's also are less prone to stomach cancer. There is also an environmental correlation, the incidence of stomach cancer increasing from south to north in Britain; this, however, must depend on some quite other factor than blood-grouping, since in fact the frequency of A in the British population *falls* as we pass northward (see Roberts, 1953, 1954*b*).

Sheppard (1953) has suggested that further analysis might show that susceptibility to stomach cancer was associated with a combination of A with the secretor gene, which would lead to large quantities of the A substance being swallowed. I understand from Professor Aird that this suggestion is now being followed up, and that data are also being collected on the relation of blood-group morphism to cancer of the colon and breast, to brain tumours and to pernicious anæmia

and the leukæmias. The results already show the same differential between A and O as regards cancer of the colon.

Meanwhile, Aird and his team have established an even more striking differential effect in peptic (gastric and duodenal) ulcers (Aird, Bentall and Mehigan, 1954). Here the selective differential between O and A is reversed, O's being much more prone to ulcer than the average of the general population, A's and B's much less so: "persons of group O are almost 35 per cent. more likely to develop peptic ulceration than are persons of the other groups". This has been confirmed by Wallace (1954). It is interesting and curious that B's are at a selective advantage both in regard to stomach cancer and peptic ulcers. Aird suggests that the mucopolysaccharide antigenic blood-group substances may be concerned in these results, perhaps by some kind of protective action against carcinogens and against factors such as hyperacidity which promote ulceration.

This 35 per cent. excess proneness of O's to peptic ulceration is a very large differential, and must have considerable selective effect on viability and general fitness in the biological sense.

To sum up from the genetic angle, we find that the alleles of the ABO morphic system are all ambivalent in respect of one or other character, conferring selective advantage in some respects, selective disadvantages in others; and very curiously, the ambivalence is often in the opposite sense in the two sexes. As previously indicated, such selective ambivalence will lead to the establishment of a morphic balance. Furthermore, the selective advantages and disadvantages of the various morphs tend to cancel each other, so that the morph-ratios in any given population are more or less stabilised.

I now come to perhaps the most striking example of morphic balance in man, and the only one in which an extrinsic selective advantage as well as an intrinsic disadvantage of the morphic system have been clearly established. Sickle-cell anæmia is a hæmolytic blood-disease, often fatal and almost always resulting in reproductive failure, characterised by shrunken erythrocytes in which the hæmoglobin is chemically different from normal, being relatively insoluble when reduced. In single dose, the sickling gene produces no visible effect on the blood corpuscles *in vivo*, but causes sickling in blood reduced by being incubated anaerobically: this condition has been called "sicklemia" and those possessing it "sicklers". Further, it is not only not fatal, but possesses the advantage of conferring protection against subtertian malaria (Allison, 1954; Anon., 1954*b*). This, of course, will only be of selective value in strongly malarious districts. In such districts, sicklers show a significantly lower incidence of malaria than non-sicklers and are less easily infected artificially; the frequency of sicklers also increases with age, showing a differential death-rate of non-sicklers.

The sickling gene only occurs in populations from areas where malaria is endemic; it is found among African negroes (in decreasing frequency from east to west), in the Veddoids of South India, the

probable Veddoids of South Arabia, and in Greece and southern Italy (Lehmann, 1954). The quantitative correspondence between sickle-gene frequency and degree of endemicity of malaria is not accurate (Moore *et al.*, 1954; Roberts and Lehmann, *Brit. Med. J.*, 1955, p. 519); this may be due to recent migration or to modifiers, but does not affect the general correlation.

Pauling and his associates (1949) have provided the biochemical and genetic clue to the ambivalence of the sickling gene. It determines a chemical alteration of the hæmoglobin (probably in its globin) which apparently alters the surface properties of the molecule in such a way as to lead to birefringence and to crumpling at low oxygen-tensions. In homozygous sicklers, there is no normal hæmoglobin, and the erythrocytes are destroyed, leading to severe and often fatal anæmia. The sickling gene is often described as dominant, but it is actually what I have called a *diversifier* (p. 26). Both it and its normal allele produce their characteristic effects when co-present; the heterozygous sicklemic thus has erythrocytes containing both normal and abnormal hæmoglobin. We may presume that the normal hæmoglobin is sufficient to prevent serious anæmia, while the abnormal is sufficient to render the blood-corpuscles "unpalatable"—unutilisable or uninfectable by the malaria parasite.

In North American negroes sickle-gene frequency is much lower than in Africa. This is presumably in part due to the fact that "negroes" in the U.S. are largely hybrids with whites, but also, as Allison suggests, to the fact that most of the area is non-malarious, which will reduce the selective advantage of the sickling gene and lead to a reduction in its frequency.

It was at one time thought that the mortality of homozygotes was higher in American than in African negroes, which could be explained on the assumption that in its original home the deleterious effects of the sickling gene had been buffered by protective modifiers, and that the buffering system had been largely destroyed by race-crossing in the U.S. (*cf.* the "de-buffering" of the deleterious hyperpituitarism of St Bernard dogs on crossing with Great Danes (Stockard, 1941) and the deleterious effects of *Platypoecilus* macromelanophore genes in species-crosses, mentioned on p. 18. However, Dr Allison tells me that the difference is in all probability apparent only, due to inadequate medical statistics in Africa. Neal *et al.* (1951) have shown that heterozygotes enjoy higher biological fitness.

The position is further complicated by the existence of other alleles causing less extreme symptoms (hæmoglobin C; Edington and Lehmann, 1954), and probably of modifiers affecting the proportion of abnormal hæmoglobin in heterozygotes (Allison, *in litt.*). Lehmann and Edington (1954) suggest that even homozygotes enjoy some advantage in protecting against malarial symptoms. Roberts and Lehmann (op. cit.) give data on the distribution of four hæmoglobin morphs in Africa.

In any case, sickling provides an outstanding example of how a

mutant which might be regarded *a priori* as essentially deleterious, may in certain circumstances possess a selective advantage, become ambivalent, and so form the basis for a balanced morphism.

Thalassemia is another genetic blood-disease which gives a rather similar picture. It depends on a single, partly dominant gene, which inhibits the formation of adult hæmoglobin. In double dose it causes Cooley's disease and is lethal before reproductive age is reached, and in single dose produces symptoms ranging from clinically healthy microcythæmia, through mild to severe but not lethal microcytic anæmia. It occurs in morphic balance, with frequencies up to 10 per cent., in malarious areas of Italy and Greece, and also (Allison *in litt.*) in Thailand, where it presumably arose independently. Allison suggests that the heterozygotes may prove to have a greater resistance to malaria, as with the sickle-cell gene : and Bianco, Montalenti *et al.* (1952) have shown that they show a higher fertility than the homozygous normals, even though the mortality of their children is higher. The total number of conceptions per family, including abortions and stillbirths, is 5·89 for heterozygotes as against 3·60 for normals ; and the total number of living children found was 3·26 as against 3·14. Further statistics on this interesting phenomenon would be of great interest.

I now pass to cases, some of them very curious, where no selective effects have yet been discovered. Some of these involve visible differences, either structural, *e.g.* lobed versus non-lobed ears ; or behavioural *e.g.* position of the crossed hands, whether with right or left thumb uppermost, etc. ; tongue-rolling, or the ability versus inability to roll the protruded tongue into a trough (Sturtevant, 1940 ; Komai, 1951). Komai (op. cit.) has found a gene in Japan which may be quite widespread and prevents full extension of the tongue, and also renders difficult the playing of wind-instruments and the pronunciation of the letters r and l. These last cases are of wide interest, since ability to control tongue-movements might well be correlated with ability to pronounce certain sounds. Darlington (1947, and see Darlington and Mather, 1949, p. 361 ff.) has already demonstrated a correlation between another human morphism, in this case high frequency of the O blood-group allele, and the existence of fricative dental (th and dh) sounds in the language spoken. Much interesting research obviously remains to be done on the differential ability of individuals of different genetic, and especially different morphic constitution, to pronounce various sounds.

Perhaps the most interesting human morphisms concern sensory capacity. Thus Muller has pointed out that the widespread existence of myopia in so many human societies, which is usually ascribed to the absence of counter-selection and the consequent accumulation of the loss-mutants determining it, could be, in part at least, explained as the result of positive selection, myopic individuals being at an advantage in the performance of fine work demanding close vision. This selective advantage would only begin to operate at a certain stage in human cultural evolution, and would cease with the widespread use of

spectacles ; but selection over this period of 6000 or 7000 years would be long enough to effect a considerable increase in myopic frequency. (See Sorsby, 1951, for facts concerning variations in the frequency of myopia.)

The most familiar human sensory morphism is red-green colour-blindness, whose frequency is far too high to be due solely to recurrent mutation. The commonest gene concerned is a sex-linked recessive, and the frequency of affected males (and therefore also of carrier females) varies between 3 and 8 per cent. in most races, though it is only 1 per cent. or lower in Eskimos and Navaho Indians. Other minor defects of colour-vision appear also to depend on sex-linked genes (Pickford, 1953), which suggests the existence of a morphic system of linked genes all concerned with this character. However, we know nothing as yet of the selective advantage of the mutant morph, which must be operative. In insectivorous birds, Ford (*in verbis*) has suggested to me that red-green colour-blindness might be of marked advantage in detecting insects which have evolved cryptic colouration in relation to predators possessing normal colour-vision. Thus normal human beings find great difficulty in detecting the larvæ of the Emperor Moth (*Saturnia pavo*) against their normal background of blossoming Ling (*Calluna*) ; but red-green colour-blind persons readily pick them out, even at a considerable distance, by their shade-differences.

Another sex-linked sensory morphism has recently been discovered —the ability to smell solutions of KCN. Eighteen per cent. of white Australian males are " non-smellers " for this substance (Kirk and Stanhouse, 1953). Dr Courtney-Pratt (*in litt.*) writes that marked variation in threshold exists for the " vile smell " of phenyl isocyanate. Hunt (1953) showed that weak kerosene increased the olfactory sensitivity of blowfly larvæ to NH_3.

The best-analysed sensory threshold morphism is that for tasting phenylthiocarbamide (PTC). " Tasters ", with low threshold, include a majority from about two-thirds to nearly 100 per cent. of human populations, but the frequency varies somewhat with sex and ethnic composition (see Boyd, 1950). In some ethnic groups, *e.g.* North American Indians and Mongoloids, the non-tasting allele is absent except as a rare mutant. In the Vietnamese the frequency of non-tasters is distinctly lower than in whites (10-20 per cent.), though higher than in Chinese (6 per cent.) and Koreans (3 per cent.), and slightly higher than in Japanese (9-14 per cent.) (Huard *et al.*, 1953).

Non-tasting depends on a recessive allele, but modifiers exist which affect the threshold. There are also aberrant tasters, commoner in Far Eastern peoples, to whom the normally bitter PTC tastes acid, salty, sweet, like orange-peel, etc. (Huard *et al.*, op. cit.). Barrows (1947) found about 5 per cent. non-tasters of brucine, with a threshold 4000 times as high as that of tasters. Non-tasting is probably recessive.

PTC morphism also must be very ancient, since it occurs also in chimpanzees and other anthropoids, and with a similar minority

of non-tasters (Fisher, Ford and Huxley, 1939). A minority of non-tasters also exists in rats (see Boyd, 1950, p. 282), who suggests that "tasting" here was originally correlated with ability to taste the chemically related but physiologically important substance, thiouracil.

Blakeslee (1935*a* and *b*) has discovered a number of other sensory threshold morphisms, both for smell and taste. Thus over 25 per cent. of people can taste benzoate of soda, advertised as a tasteless food-preservative! I have already mentioned that in about 20 per cent. of tasters, PTC has other tastes than the normal. In some cases, there are two components in the taste of one substance (*e.g.* mannose), or in the smell of one strain of flowers, *e.g.* Freesias. In such cases some people can smell or taste both, some the first only, some the second only, and some neither. This clearly implies the involvement of two independent morphic genes. We may expect sensory threshold morphism to be widespread in higher animals: the devising of suitable methods for their detection in other groups than mammals will open up an interesting field of research.

Since sensory threshold and blood-group morphisms can be readily investigated on a large scale in man, we may expect that their study will help materially in the mapping of the human gene-complex and the elucidation of ethnic relationships. In any event, morphism in man is likely to prove the most fruitful area of human genetics. It has disclosed new and surprising phenomena, and will undoubtedly lead to new approaches in general genetics. It is making it possible for man to repay his genetic debt to other organisms. Animals like *Drosophila* and fowls, plants like *Primula* and maize, have enabled us to lay the foundations of human genetics. Now man is beginning to shed new light on the genetics of the rest of the organic world.

7. CONCLUSION

It will now be apparent that morphism (balanced genetic polymorphism) constitutes an important mode of intraspecific differentiation in many groups of animals and plants. In certain cases it may give rise to secondarily monomorphic populations, which, if isolated, may then evolve into distinct species or subspecies. It may involve a great variety of characters, both extrinsically adaptive (such as mimetic or cryptic resemblance, disease-resistance, temperature-tolerance, clutch-size and migratory habit in birds, germination-time in plants, caste in social insects) and non-adaptive (such as demonstrably non-allesthetic characters of colour or pattern). Its genetic basis may be genic (one or more single genes or groups of closely linked genes); or chromosegmental (inversions or translocations); or chromosomal (one or more extra chromosomes); or a high degree of continuous variance affecting meristic characters or stages (number of eggs in birds or moults in arthropods).

But we still do not know the details of its distribution in any given phylum or class; why some sub-groups (*e.g.* in birds and mammals) show frequent visible non-adaptive morphism and others show none;

why some groups (*e.g.* in Diptera) show abundant inversion-morphism but few or no phaneromorphic characters; why other groups show mainly or only genic morphism; or why (and where) morphic differentiation sometimes gives rise to secondary monomorphic differentiation. We are lamentably ignorant of the nature of the selective forces involved in most morphisms, and of the genetic stability-mechanisms underlying them. And in many cases we do not know whether polymorphic variation in a species is due to true morphism or to recombination after crossing of geographically differentiated monomorphic types, or even to geographical differentiation alone.

I have the feeling that Bateson would have enjoyed both our knowledge and our ignorance concerning morphism and would have found satisfying occupation in the study of the many problems which it poses.

Every clear case of true morphism challenges the general biologist to discover the ecological influences, the selective forces, and the genetic mechanisms at work. And the realisation of the widespread significance of morphism as the basis for a special type of intraspecific adaptive differentiation points to the need for a concerted and comprehensive survey of its distribution and role in all groups of animals and plants.

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ADDENDA

The page-references refer to the relevant pages of the main paper

Page 5. Jamieson (unpub.) suggests that there may be genetic differences in responsiveness to the stimuli inducing the two " phases " of *Locusta migratoria.* If so, the dimorphism would be partly genetic though mainly environmental.

Page 7. For further theoretical discussions bearing on " heterosis " as a basis for morphism, see Buzzati-Traverso, Dobzhansky, and other articles in Gowen (1952) ; Tebb and Thoday (1954) ; Burdick, 1954 ; Mather (1955), and especially Stone, Alexander and Clayton (1954), with its interesting discussion in biochemical and developmental terms. Sang (1955) has demonstrated the greater biochemical and metabolic efficiency of heterozygotes between two highly inbred lines of *Drosophila*, but this particular form of heterosis could probably not be utilised as a genetic basis for balanced morphism, as it presumably depends on a number of freely segregating genes.

Page 14. Interesting symmetry morphisms are found in Flatfish (Norman, 1934 ; Hubbs and Hubbs, 1945 ; Ginsburg, 1952). Most groups are essentially monomorphic dextral or sinistral, but some species are dimorphic for asymmetry. The normally dextral European Flounder, *Platichthys flesus*, has 5-36 per cent. sinistrals, the ratio varying geographically. The American *P. stellatus* is about 50 per cent. sinistral in California, 75 per cent. in Alaska, and 100 per cent. (monomorphic) in Japan. Most species of *Paralichthys* are 100 per cent. sinistral, but *P. californicus* and *P. aestuarius* are nearly 40 per cent. dextral. Some species of *Hippoglossina and Xistreuris* are " indifferently " dextral or sinistral, as in the primitive genus *Psettodes. Psettodes* is also dimorphic for the asymmetry of its optic chiasma ; but higher forms are monomorphic (*either* dextral *or* sinistral) for this character, even in specimens with reversed symmetry of body-form. Reversed individuals, *e.g.* in *P. flesus*, appear to be at a selective disadvantage, as their frequency decreases

with age. A further analysis, aimed at discovering the counterbalancing advantage of reversal, would be of interest.

In the worm, *Spirorbis*, and the mollusc, *Ancylus*, monomorphic, sinistral and dextral species occur, but no species dimorphic for asymmetry (Caullery and Mesnil, 1897 ; Holmes, 1899).

Page 13. Some Deermice (*Peromyscus*) show colour-morphism. *P. maniculatus* is dimorphic buff or grey in at least 4 subspecies, buff being dominant (Clark, 1938). In *P. m. blandus*, buffs vary from 24 to 57 per cent., apparently in relation to their cryptic value on soils of different tint (Blair, 1947).

The common Water-vole of Britain, *Arvicola amphibius*, has a melanic morph whose frequency increases to the north (the " normal " grey-brown morph of the northern subspecies *A. a. reta* is also darker). In addition, all-black colonies of *A. a. amphibius* occur in the Fen district of E. Anglia (Matthews, 1952, p. 158).

The Stoat, *Mustela erminea*, is well-known for its seasonal dimorphism, turning white in winter. However, the *capacity* for this seasonal colour-change exhibits a marked ratio-cline, with more winter-whites to the north and east, and hardly any in the Irish sub-species *M. e. hibernica*. While temperature and possibly day-length affect the process, genetic capacity is also clearly involved, and exhibits a geographically-graded true morphism. The British Weasel, *M. nivalis*, never whitens in Britain but does so in northern and mountainous regions in Europe (Matthews, 1952, pp. 225-7, 251-2).

The Brown Rat, *Rattus norvegicus*, has a melanic morph sporadically all over its range, while the Black Rat, *R. rattus*, shows geographical colour-variation which has led to hybrid recombinational variation in certain areas (Matthews, 1952, p. 169).

The White-throated Packrat, *Neotoma albigula*, is dimorphic, with melanics outnumbering " normal " greys in areas of dark lava (Blair, W. F., 1954, *J. Mammal.*, *35*, 239). The advantage of the melanic is cryptic ; the presence of about 33 per cent. greys is due to gene-flow and immigration from non-dark areas.

The mongoose, *Herpestes brachyurus*, is dimorphic (reddish and blackish) in several subspecies (Schwartz, 1947). Stullken and Hiestand (1953) show that pigmentation may be of general (metabolic) advantage in relation to heat-conservation.

Page 16. Melanics occur in the introduced *Trichosurus vulpecula* population of New Zealand (Wodzicki, 1950), but whether they were part of the original introduction or due to the spread of later mutants is uncertain ; nor are any data as to morph-ratios available. Wodzicki does not record any colour-variants in the introduced Rabbits in New Zealand, and mentions the extreme rarity of winter-whites in introduced Stoats in New Zealand. It would be interesting to determine the incidence of morphism in introduced species, and the residual polymorphism (if any) in feral populations of variable domestic species such as cats and pigs.

Page 18. Bateson (1913) gives various examples of sharp coloration-morphism in insects. *E.g.* the yellow-spotted butterfly, *Thais rumina*, has a red-spotted morph in one area (p. 27) ; the moth, *Noctua castanea*, is dimorphic for red and grey forewings, with geographical variation in morph-ratio : in one British locality, a rare yellow-winged morph occurs (p. 122). The high polymorphism of the Potato-beetle, *Leptinotarsa*, later described by Tower (1918), appears to be largely geographical, but with true morphism also involved : it might well be re-investigated by modern methods. The Lesser Underwing Moth, *Tryphaena conus*, has a dark morph (*curtisii*) in the northern parts of its range. Ford (1952) has shown that the *curtisii* morphs in Orkney and in the Hebrides, though indistinguishable by inspection, have had their dominance independently established by the incorporation of different modifiers.

Page 19. *Lebistes* is much more ecologically versatile than the related (and often sympatric) *Poecilia vivipara* and *Micropoecilia parae*, which are monomorphic

in both sexes (*cf. Drosophila*, p. 12). *Platypoecilus* (now *Xiphophorus*) *maculatus* has numerous morphic patterns in both sexes. These are controlled by two sets of multiple alleles (or semi-alleles), one (autosomal) of 7, the other (sex-linked) of 5, for micro- and macro-melanophore pattern respectively. The morph-ratios differ considerably in different isolated populations, and some alleles are absent in most. One population has had a roughly constant morph-ratio since 1867. The related monomorphic *P.* (*X.*) *couchianus* and *P.* (*X.*) *xiphidium* are more restricted in range (Gordon, 1947; Gordon and Gordon, 1950, 1954; Clark, Aronson and Gordon, 1954). Myers (1925, 1931) states that *Mollenesia*, *Gambusia* and *Fundulus* may show "melanodimorphism".

In the Bluehead Wrasse, *Thalassoma bifasciatus*, Tee-Van (1932) showed that most of the striking variation is physiologically determined, but some seems to have a genetic (morphic) basis. Pomini (1940) claims that in trout (*Salmo trutta*), in addition to marked geographical polymorphism, some populations show true morphism. Hardy (1955, *The Open Sea*, Collins) states that the marine catfish, *Anarrichas lupus*, has a spotted morph in addition to the usual banded form.

Page 20. In *Primula sinensis* short-style homostyly depends on a gene which enlarges the "eye" of the corolla as well as reducing style-length in genetically long-styled plants—an excellent example of a "correlated character" (p. 13). For references and other examples, see Huxley, 1942, p. 189.

Page 21. Excessive variability, presumably implying continuous morphism, occurs in many other animals: *e.g.* in the moths, *Dianthoecia carpophaga* (as against the "monomorphic" *D. capsincola*), *Agrotis segetum* and *A. tritici* (as against *A. suffusa* (*ypsilon*)), *Noctua festiva* (as against *N. triangulum* and *N. umbrosa*), *Taeniocampa instabilis* (as against *T. cruda* (*pulvurulenta*)), *Plusia interrogationis* (as against most other species), or *Peronea cristata* (as against *P. schalleriana*); see Bateson, 1913, ch. 1.

Page 23. Hoestland (1955, *C. R. Acad. Sci.*, *240*, 916) gives further data on *Sphaeroma*. In Britain, as in Brittany, it has five "structural" and two coloration morphs. The frequency of most morphs is correlated (to varying degrees) with temperature; but of one, with sheltered habitat. Morph-ratios differ in the various islands of the Scillies.

Page 25. Callan (1941) discovered a sex-chromosome morphism in the earwig, *Forficula auricularia*, males being either X_1Y or X_1X_2Y, those of the latter type producing fewer male than female offspring. Callan suggests that this may be an advantage where population is dense, and one male can accordingly fertilise several females. In any event, the male sex-ratio does vary markedly in *Forficula*, and is lowest (16 per cent.) in the very dense populations of the Scilly Islands (Brindley, 1912), where the greatest proportion of "high" (long forceps) males also occurs (p. 25). Further research on this association of two morphic characters would be of great interest.

Page 34. Grüneberg (1947) refers to various blood-group systems in other mammals. Thus, in domestic rabbits, there are several, including one whose genetic basis (3 multiple alleles or semi-alleles) is similar to that of the human ABO system. In rats there are 4 blood-groups; and in wild *Peromyscus* there are specific and subspecific antigenic differences. The high correlation (0·7) found between leucocyte-level and longevity in rats and mice shows how blood-characters may have selective correlates. He also mentions genetic differences in disease-resistance in various animals, in resistance to various drugs in mice, and in metabolic capacity in rabbits and sheep, which presumably might in certain circumstances become established as morphisms.

Haldane (1954, ch. 2) gives examples of genetic biochemical differences in blood and other characters which may prove to be morphic.

SELF-INCOMPATIBILITY SYSTEMS IN ANGIOSPERMS
III. CRUCIFERAE

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1. INTRODUCTION

THE demonstration that self-incompatibility in *Iberis amara* is determined by a multiple-allelic system with sporophytic determination of both style and pollen, and independence, dominance and mutual weakening (Bateman, 1954) naturally raises the question whether similar systems operate in the many other self-incompatible Cruciferous species. Several of these have been studied previously though the interpretations have not generally been in accord with my own conclusions in *Iberis*.

It therefore seemed desirable to re-examine the data on these other species to see whether alternative interpretations are possible which would bring them into line with the *Iberis* results.

2. *CARDAMINE PRATENSIS*

This species is of special historical interest because it was the first homomorphic species to be the object of a genetic study (Correns, 1912). The progeny of two plants intercrossed fell into four classes of equal size: compatible with both parents, compatible with the first parent, compatible with the second parent, and compatible with neither (see fig. 1). Correns' hypothesis assumed sporophytic control of pollen and style with dominance and independence. His parental genotypes were taken to be **Bb** and **Gg** and the progeny: **BG** (incompatible with both parents), **Bg** (incompatible with **Bb** only), **bG** (incompatible with **Gg** only), and **bg** (compatible with both parents). Thus the big letters were dominant over the little letters but were independent of one another. Correns was not completely satisfied with his hypothesis and did not press it very hard. Indeed, it broke down when he intercrossed plants from the four sib classes, for, as shown in the figure, these four classes all appeared to be heterogeneous. It may be pointed out that, however heterogeneous a population may be, one can never distinguish more than four classes, if they are recognised only by two alternative reactions in two tests (*i.e.* as compatible or incompatible with two tester plants, the parents).

Fig. 1 summarises Correns' results, showing firstly the reaction when the sibs were tested with their parents' pollen (the basis of the classification into mating groups), and secondly, the results on intercrossing. While there is in fact a correlation between expectation and observation (out of 664 pollinations 526 agreed with expectation and

138 disagreed) the discrepancy is too great for one to accept Correns' explanation unmodified. Neither he, Beatus (1934), nor myself have succeeded, however, in producing a completely satisfactory modification of the original hypothesis.

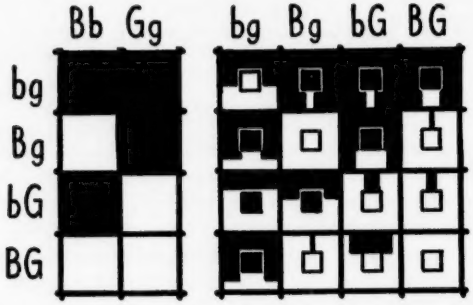

FIG. 1.—Diagrammatic representation of Correns' results with *Cardamine pratensis*. Columns, plants tested by pollen : rows, plants tested by style. Black, compatible : white, incompatible. Plants are represented by their presumed genotypes. **Bb** and **Gg** are the parents ; **bg, Bg, bG** and **BG** are the offspring, classified into four groups according to their compatibility with their parents' pollen (left-hand block). The right-hand block shows : smaller squares, the expected results on intercrossing sibs ; and larger squares, the results obtained. The relative frequencies of compatible and incompatible pollinations are shown by the relative areas of black and white in each square.

The acceptance of Correns' hypothesis by geneticists generally was further handicapped by the confusion in his mind between recessivity and inactivity, so that there was much discussion on how the **bg** genotype could be self-sterile. This would seem to be a product of the " presence-and-absence " theory of dominance.

Then came the analysis of *Nicotiana* by East and Mangelsdorf (1925) which was soon shown to apply to many species, and overshadowed Correns' work. Indeed it came to be accepted as the *only* system of homomorphic incompatibility.

One further attempt to confirm Correns' results in *Cardamine* was made by Beatus (1934) but although he tried to incorporate polyploid inheritance into his analysis the amount of pseudo-compatibility was too high to allow of any definite conclusions.

In the meantime, Lawrence (1930) showed that the behaviour of Correns' four classes on backcrossing to the parents (though *not* the crosses between sibs) could be explained on East's hypothesis by assuming tetraploidy and an unexpectedly high concentration of *S*-alleles (the parents being **S1 S1 Z1 Z2** and **S3 S3 Z3 Z4**). Thenceforth Correns' own hypothesis was disregarded.

However, *Cardamine pratensis* is particularly unsuitable material for a critical analysis since there are two common races in Northern Europe, one with $2n = 30$ (a secondary tetraploid from $x = 8$) and the other $2n = 56$ (a secondary octoploid). The octoploid is the

more widespread and it is very probable that it was this form with which Correns dealt. A cross between plants with no common allele would then produce a family with 64 different genotypes and a maximum of 64 mating groups. It is not surprising therefore that Correns' four initial groups appeared heterogeneous on further testing ! One can, however, conclude that the general nature of Correns' results are in accord with sporophytic control of the pollen, as he first postulated. This applies in particular to the incompatibility between parent and progeny and the high degree of cross-incompatibility between sibs (368 out of 664 pollinations).

3. *CAPSELLA*

This genus has one self-incompatible species, *C. grandiflora*, which was the subject of intensive study by Riley (1932, 1936). It has since been regularly cited as an anomalous species, falling between heteromorphic di- or tri-polar incompatibility (*Primula*, *Lythrum*) and homomorphic multi-polar incompatibility (*Nicotiana*, *Trifolium*) : (see Bateman, 1952). The first has sporophytic control of the pollen, the second, gametophytic.

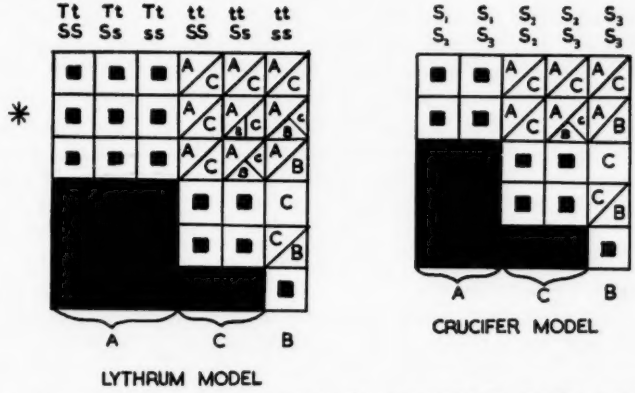

FIG. 2.—A comparison of the genetic models of incompatibility in *Capsella grandiflora* as proposed by Riley (*Lythrum* model) and myself (Crucifer model). A, B and C are the three mating groups. The proposed genotypes are shown at the head of the columns (pollen parent). The same genotypes in the same order are represented in the rows (seed parents). Dotted squares, incompatible pollinations : solid squares and those with letters entered, compatible pollinations. The size of the compartments allotted to each letter represent the proportions of those mating groups to be expected in the progeny. The genotype **TtSs**, shown by an asterisk, in the *Lythrum* model, has no counterpart in the Crucifer model. **T-t**, **S-s** are Riley's own gene symbols.

Riley established beyond doubt that the *Capsella* system involved sporophytic control of both pollen and style, with dominance. This (apart from Correns' neglected work) was sufficiently novel at the

time, and Riley sought for already familiar systems which might fit his data. These he found in the tristylic *Lythrum* and *Oxalis*. To account for the presence of only three mating groups, two loci were invoked, each with two alleles, showing dominance and epistasy. Riley's hypothesis differed from *Lythrum* only in morphology, since *Capsella* was homomorphic.

In view of what we now know of other crucifers it appears opportune to re-examine Riley's evidence to see whether alternative explanations would fit.

As shown in the first paper of this series (1952) the three mating groups of trimorphic species due to two loci with two alleles at each, can be closely simulated by a single locus with three alleles in a dominance series **S1** > **S2** > **S3**, common to both sexes.

Fig. 2 compares the two hypotheses. Riley's **T** is epistatic to **S-s** so that all **T**-carrying plants are incompatible and **TT** plants cannot arise from compatible matings. Similarly **S1S1** plants are not present in my model. In both models there are only three phenotypic mating groups which have been called (following Riley's notation) *A*, *B* and *C*, but in Riley's model there are six genotypes, in mine only five. Each of my five genotypes behaves exactly the same as one of Riley's, both in compatibilities and in the groups produced from compatible matings. But the sixth genotype in Riley's model, the double heterozygote, **Tt.Ss** has no counterpart in mine. It behaves as the exact intermediate between **Tt.SS** and **Tt.ss** and its most distinctive feature is that when crossed to group *B*, the bottom recessive, it produces a 3-group family (2*A* : 1*C* : 1*B*). The most critical test for deciding between the two models would therefore be the presence of a 3-group family in crosses between groups *A* and *B*. One such family, and one only, was obtained : family 3342 in table 1 of the 1936 paper. The observed frequencies were, however, 1*A* : 9*C* : 10*B*, when the expectation was 10*A* : 5*C* : 5*B*. χ^2 for deviation from expectation is 16·3 for 2 degrees of freedom ($P = < 0{\cdot}001$). The most likely explanation therefore is that the family was of type 1*C* : 1*B* with a single *A* contaminant. While one of the parents of this family was a known *B*, the other was only inferred to be *A* because of the presence of *A* in the family. It seems likely that it was in fact a *C*.

Every other of the 49 families analysed by Riley would give the same expectations in either model. By analogy with *Iberis* and *Cardamine* it would appear most likely, therefore, that *Capsella* also has one locus with at least three alleles showing dominance. Riley himself recognised that since his material was inbred the presence of only three mating groups did not imply that the whole species was restricted to three groups. If any further groups did arise, the Cruciferous scheme could accommodate them easily whereas the *Lythrum* scheme could not.

4. *BRASSICA*

Kakizaki's paper (1930) on self-incompatibility in *B. oleracea* is constantly referred to in discussions on the subject. The elaborateness of the hypothesis, evolved to explain results which have not since been confirmed, is enough to make one doubt its validity. Kakizaki's experiments were largely concerned with self-fertility which in general is quite rare in cabbages (though the author understands from F. R. Horne, Director of the National Institute of Agricultural Botany, that it is very common in cauliflower). A recent account by Odland and Noll (1950) stated that of 1000 plants of Pennsylvania State Ballhead, only four were pseudo-compatible and none fully self-compatible. These authors also quote four earlier papers by U.S. workers to the same effect. So Kakizaki's sympathetic **T** factors (promoting self-fertility; no relation to Riley's factor with the same symbol) could not in any case be of great importance to the species.

Ten plants were selected from each of two cabbage varieties and studied separately. In each sample Kakizaki concluded that there were the same three oppositional alleles (**S1**, **S2**, **S3**) and the same two sympathetic alleles (**T1**, **T2**) (there was no cross-testing). Plants heterozygous for their **S**-alleles were self-sterile unless they were at the same time homozygous for a **T**-allele which was then epistatic (in the style) to the **S**-allele. The **T**-alleles promoted the growth of pollen only in styles homozygous for the same **T**-allele. Plants homozygous for an **S**-allele were *always* self-sterile. Both **S** and **T** acted at the gametophytic level in the pollen.

Thus we have:

Double heterozygotes; **S1 S2 T1 T2** self-sterile.
Homozygotes for **T** only; **S1 S2 T1 T1** self-fertile.
Homozygotes for **S**; $\begin{pmatrix} \mathbf{S2\ S2\ T1\ T2} \\ \mathbf{S2\ S2\ T1\ T1} \end{pmatrix}$ self-sterile.

Such a system certainly agreed with the results, which were:

(*a*) High cross-incompatibility between unrelated plants.
(*b*) Different reactions of two cross-incompatible plants with a third plant.
(*c*) Frequent reciprocal differences in compatibility.
(*d*) Self-fertility.
(*e*) Incompatibility of some self-fertile plants with some self-sterile plants.
(*f*) Presence of self-sterile plants in progeny of self-fertiles.
(*g*) Presence of self-fertile plants in progeny of self-steriles.
(*h*) Cross-incompatibility of two self-fertile plants.

Now Kakizaki never presented proof of gametophytic control of the pollen. He merely followed East in assuming it. From our present knowledge of the Cruciferae it would seem likely that the pollen was

in fact sporophytically controlled. In which case the results can be readily explained, without need of sympathetic factors. Results (*a*), (*b*) and (*c*) are characteristic of sporophytic control with dominance and independence. Results (*d*), (*e*), (*f*) and (*g*) have already been experienced in *Iberis amara* (Bateman, 1954) where they appeared

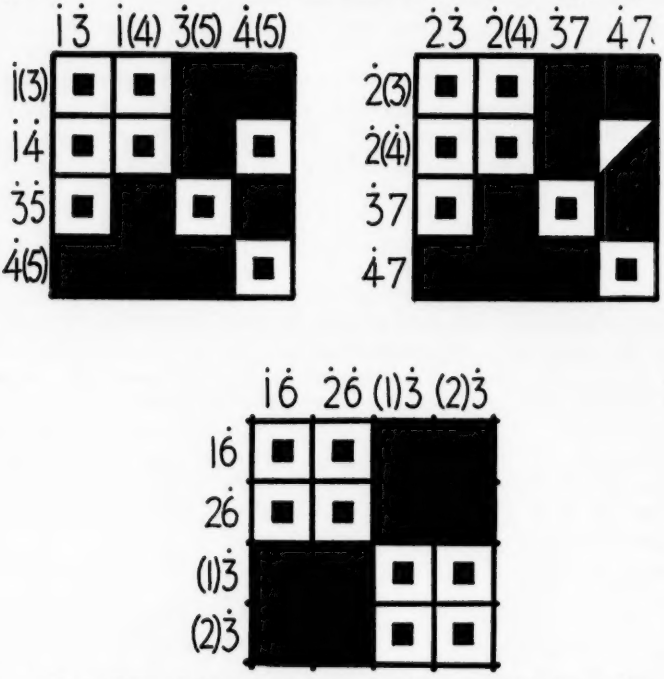

FIG. 3.—Compatibility relationships found in three typical families of *Iberis amara* (Bateman, 1954). Dotted squares, incompatible : solid squares, compatible : half-filled squares, partially compatible. The numbers represent the S-alleles (S1-S7) with their presumed activities indicated as follows :

i active	(4) partially active
(4) inactive	7 activity unknown

The alleles of the third family are not the same as the alleles with the same numbers in the other families.

to be due to an allele S_f which was recessive to some active S-alleles and dominant to others. Alternatively there might be a weakening of the reaction in plants heterozygous for particular S-alleles which interacted in a manner envisaged in the first paper of this series.

Point (*h*) does present a difficulty. On Kakizaki's interpretation it arose on crossing self-fertiles of the constitutions S1 S3 T1 T1 and S1 S3 T2 T2. In the actual data, however, only a single pair of plants was involved and some seed was obtained in the reciprocal crosses though not as much as on selfing. But as no emasculations were made

and *Brassica* is automatically self-pollinating, seed yields consistently less than those obtainable on selfing should be impossible. The low seed yields probably arose from causes other than incompatibility.

Summarising, the assumption of sporophytic control permits an adequate explanation of all Kakizaki's results with one dubious exception. Besides being unnecessarily complicated the **T**-factors have the additional disadvantage that if they were present throughout the species, self-fertility would be much more frequent than it is generally found to be.

Before discovering the suitability of *Iberis amara* for incompatibility studies I had been working with two forms of *Brassica campestris*; one turnip, the other an annual form, and with radish (*Raphanus sativus*). None of them were very suitable material for critical work because of the large amount of pseudo-compatibility. After vain attempts to fit them to a *Nicotiana* type scheme the data were put on one side.

Re-examination of the data in the light of the *Iberis* work demonstrates that they do fit into what we can now recognise as the Cruciferous type. [Correns' distinction between "Cruciferen-Typus" and "Personaten-Typus" (1928) seems justified after all in spite of the rather weak grounds on which it was originally based. We now see the terms as too narrow since the Cruciferen-Typus includes the Compositae at least, and the Personaten-Typus includes the Rosaceae and Leguminosae.]

Fig. 3 shows typical compatibility patterns between the four genotypes found in each of three sample families encountered in *Iberis* (Bateman, 1954) with the interpretation of the gene actions there proposed.

In the turnip, families produced by crosses between plants within the variety Red-top Milan showed cross-incompatibility of about 50 per cent. The larger families seemed to fall into two or three mating groups with some cross-incompatibility between groups and sometimes reciprocal differences.

The data in an annual form of *B. campestris*, grown in India as "Toria," was my most extensive prior to the investigation of *Iberis*. Since one could not be sure of the compatibility of pollinations until some weeks had passed it was necessary to plan all pollinations in advance. Short of making all possible pollinations one was bound to omit some critical tests. Reciprocal families tended to give the same mating patterns but unfortunately they were not tested against each other.

Three parent plants were intercrossed in all possible ways to give six families in 3 pairs of reciprocals. The mating pattern of each is shown in fig. 4 with the interpretations in terms of the action of the **S**-alleles on the assumption that the parent plants were **S1 S4**, **S2 S3** and **S5 S6**.

My own experience with cultivated radish had indicated a high

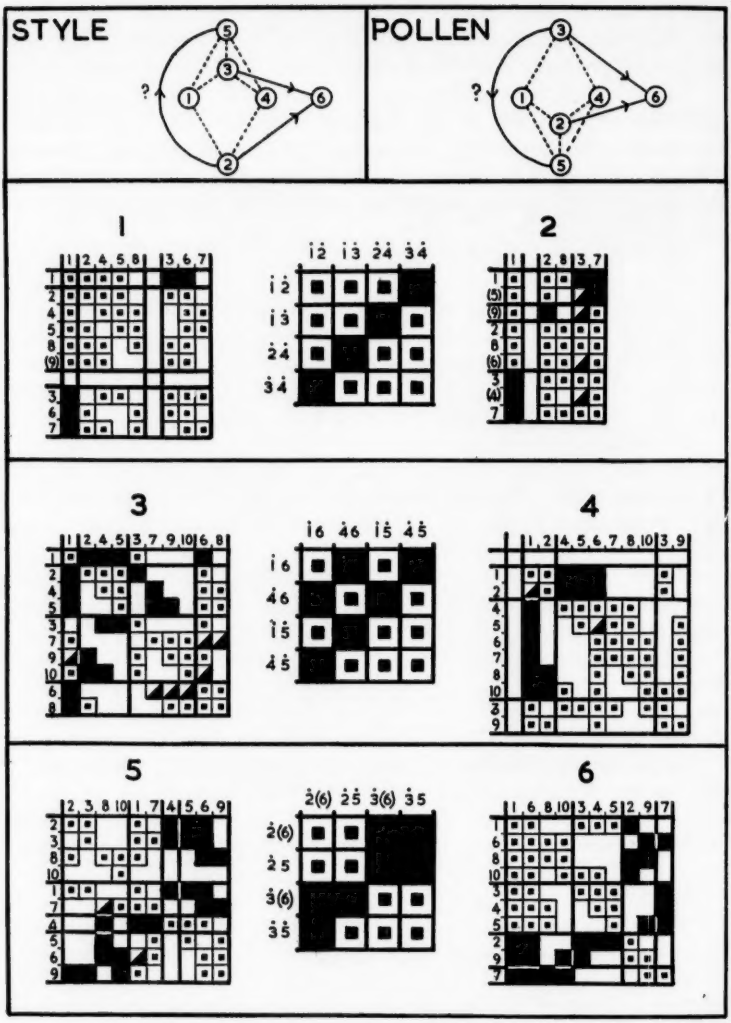

FIG. 4.—Incompatibility in *Brassica campestris*. 1 and 2, 3 and 4, 5 and 6, are pairs of reciprocal families produced by making all possible crosses between 3 plants. Symbols as in fig. 3. Pollinations not made are left blank. At left and right are the pollinations between individual sibs. Plant numbers in parenthesis are those of male-steriles which could only be tested one way. Down the centre is the interpretation of the results in terms of gene action. The interaction of the genes is shown at the top. Each allele is shown as a numbered circle. Dotted lines denote independence of action; entire lines, dominance in the direction of the arrow.

degree of cross-incompatibility between sibs (approximately 50 per cent.). Mr A. Gavin Brown, however, at the John Innes Institution made an extensive series of pollinations in 1944 and 1945 which he was unable to interpret at the time and which he has kindly allowed me to study. Viewed, now, as examples of sporophytic pollen control they are readily comprehensible. I have his permission to cite one family as an example (fig. 5).

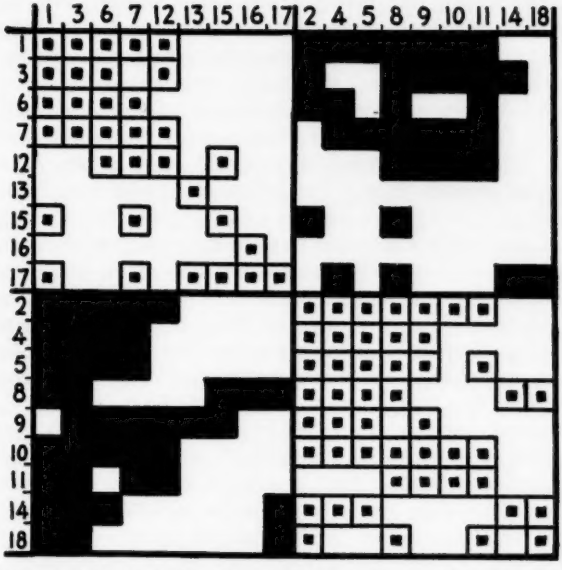

FIG. 5.—Incompatibility in *Raphanus sativus*. Progeny of a cross between single plants of two varieties, Scarlet Globe and French Breakfast. Symbols as in fig. 4.

The family was produced by a cross between two plants from distinct varieties, Scarlet Globe and French Breakfast. Unless the total number of S-alleles in the species is very small it would be unlikely that the parents should have a common allele. However, as the figure shows, this large family fell into only two mating groups which were reciprocally cross-compatible (*cf.* fig. 3).

Further evidence that sporophytic control of the pollen reaction is widespread in the Cruciferae was met with in a survey of the family which is discussed in detail later. Most of the samples were obtained as seed from botanic gardens where each species is usually maintained by few individuals. Amongst those samples which proved to be self-incompatible there was often a very high degree of cross-incompatibility. Indeed in some samples all five plants grown were cross-incompatible. This is circumstantial evidence of widespread sporophytic control.

5. TIME OF INHIBITION

According to the literature, *Brassica pekinensis* (Stout, 1931), *Capsella grandiflora* (Riley, 1936), *B. oleracea* (Sears, 1937), *Raphanus sativus* (Lewis, unpub.) and *Iberis amara* (Bateman, 1954) all show a very early inhibition of incompatible pollen. The grains barely germinate and the short, often twisted, tubes either do not penetrate the stigmatic surface at all or penetrate only a few times the diameter of the grain so that the pollen grains never empty. Not only does this similarity of behaviour suggest a common type of incompatibility system but it indicates the mechanism by which sporophytic control of the gametophytic (pollen) generation could be attained.

Sporophytic control must be determined before meiosis and persists even when the gametophyte is independent. It might be inferred, therefore, that the incompatibility resides in a persistent structure laid down at an early stage. The pollen grain wall is laid down from the cytoplasm of the pollen mother cell. If the substance responsible for the incompatibility reaction were in the wall, one can readily see that it could manifest itself immediately the pollen grain came into contact with an incompatible stigma and this would produce very early inhibition. In contrast, the determinants in gametophytic control (as in *Nicotiana*) cannot begin to operate until the completion of meiosis, and their effect would not appear until the gametophyte had developed to a certain stage.

In general then one would expect sporophytically controlled incompatibilities to show an earlier inhibition of incompatible pollen. It is of interest therefore that, in the Compositae, *Crepis*, *Parthenium* and *Cosmos* show such an early inhibition of self pollen, that it has been used as the readiest test for incompatibility by Hughes and Babcock (1950), Gerstel and Riner (1950) and Crowe (1954) in work which has demonstrated sporophytic control in that family also.

6. THE EFFECTS OF POLYPLOIDY

Polyploidy and especially autopolyploidy has often been associated with self-fertility in species hitherto self-sterile (see table in Lewis, 1949). As a result of his work with tetraploid *Oenothera organensis* (1947) Lewis concluded that this self-fertility was due to the interaction of incompatibility genes in heterogenic diploid pollen, when there had been no previous selection for their ability to work efficiently in pairs on the male side. Such an argument does not apply, of course, where there is sporophytic pollen determination, and in Crucifers, therefore, polyploidy should not be associated with self-fertility (see data of Howard, 1942).

There is an apparent exception to this rule in that in the genus *Brassica*, whilst the diploid species *oleracea*, *campestris* and *nigra* are self-sterile, their allotetraploid derivatives, *napus*, *juncea* and *carinata*, are all self-fertile.

But Mizushima (1950) reports that many artificially produced allotetraploids in the Brassiceae (including *B. carinata* and *B. juncea*) are self-sterile though with good pollen and fertile in crosses with sibs or related species. The self-fertility of naturally occurring allotetraploids must therefore be secondary. Possibly the incompatibility reaction is lost as superfluous in a species which has a new way of maintaining hybridity.

7. DISTRIBUTION OF SELF-INCOMPATIBILITY WITHIN THE FAMILY

There is no protandry, no protogyny and no dioecy in Crucifers. In most species, anthers and stigma are so placed that self-pollination is automatic, though there are exceptions (all those I have encountered have been self-fertile). Apomixis is only known in one species, the polymorphic *Arabis Holboellii*, comprising races with 2*x*, 3*x*, 4*x* and 6*x* chromosomes, in which Böcher (1951) has shown that triploids and some diploids are pseudogamous. Apart from these apomicts it is doubtful whether any species of Crucifer is totally inbreeding, for the nectaries are always well-developed and actively secrete nectar. However small and insignificant the flowers may be, their nectaries indicate that their function is to attract insects. Furthermore, in this family, the phylogeny of which has been a big problem to the systematists, the considerable diversity in nectary structure has been given special weight in determining the relationships of the genera. According to Stebbins (1951) this diversity is itself evidence of the adaptive importance of the Cruciferan nectary apparatus.

The three main factors determining the breeding system of the Cruciferae seem to be :

nectary arrangement, which will determine the route of approach of the pollinator and, to some extent, the species of pollinator ;
size of flower, which by increasing the attractiveness to pollinators will increase the amount of outbreeding ; and
self-incompatibility.

This last will be the most important. From my own observations it is strongly correlated with the second. The self-sterile species generally have the most conspicuous flowers, conspicuousness being achieved either by increased size, bright colouring or special arrangements in the inflorescence, especially pseudo-umbels (as in *Lobularia maritima* and *Iberis amara*).

A survey of the distribution of self-incompatibility in the Cruciferae will be expected to throw light on its evolutionary importance.

The data of table 1 were based, except as shown in footnotes, on material grown from seed (usually from botanic gardens). Five plants were tested in each sample. If they all set seed readily in an insect-proof house they were at once classed as self-fertile and self-pollinating. If they did not set seed freely under those conditions each was selfed by hand and crossed to the adjoining plant. If both

TABLE I

Distribution of self-incompatible species in the Cruciferae (classification according to Schulz)

SS = self-sterile : SF = self-fertile

Tribe	Genus	SS	SF	Tribe	Genus	SS	SF
Streptantheae	*Caulanthus*	...	1	Euclidieae	*Anastatica*	...	1
					Myagrum	...	1
		0	1		*Schimpera*	1	...
					Neslia	...	1
Brassiceae					*Bunias*	1	...
(Brassicinae)	*Brassica*	4	4				
	Erucastrum	1	2			2	3
	Brassicella	1	...				
	Sinapis	2	...	Lunarieae	*Lunaria*	...	1
	Hirschfeldia	1	...		*Ricotia*	1	...
	Reboudia	1	...		*Peltaria*	1	...
	Diplotaxis	3	1				
	Eruca	1	...			2	1
(Raphaninae)	*Raphanus*	1	...	Alysseae	*Vesicaria*	...	1
	Enarthrocarpus	1	...		*Alyssum*	5	4
	Crambe	2	2		*Ptilotrichum*	1	...
	Rapistrum	1	1		*Lobularia*	1	...
					Berteroa	1	...
(Cakilinae)	*Cakile*	1	...		*Clypeola*	...	1
(Vellinae)	*Carrichtera*	...	1			8	6
	Succowia	...	1				
				Drabeae	*Draba*	...	12
(Moricandiinae)	*Moricandia*	1	...		*Erophila* ‡	...	4
	Orychophragmus	1	...		*Armoracia*	1	...
	Conringia	...	1				
						1	16
		22	13	Arabideae	*Cardamine*	2	2
					Barbarea	...	1
Heliophileae	*Heliophila*	1	1		*Arabis*	2	8
					Nasturtium	...	2
		1	1			4	13
Lepidieae	*Lepidium*	...	8	Matthioleae	*Aubretia*	1	...
	Coronopus	...	2		*Matthiola*	1	2
	Cardaria	1	...		*Chorispora*	...	1
	Isatis	1	...				
	Physaria *	6	1			2	3
	Iberis	8	...				
	Biscutella	2	2	Hesperideae	*Malcolmia*	2	2
	Iondraba	1	...		*Hesperis*	1	...
	Aethionema	...	4		*Erysimum*	6	7
	Thlaspi	...	2		*Cheiranthus*	...	1
	Pastorea	1	...				
	Ionopsidium	1	...			9	10
	Teesdalea	...	2				
	Pachyphragma	1	...	Sisymbrieae	*Alliaria*	...	1
	Capsella †	1	4		*Sisymbrium*	5	5
	Hutchinsia	1	...		*Arabidopsis*	...	1
					Camelina	...	2
					Descurainea	...	1
		24	25			5	10
					Grand total	80	102

* Brewbaker (personal communication).
† Riley (1936).
‡ Winge (1940).

types of hand pollination produced abundant seed the species was considered self-fertile but not self-pollinating. If only the cross-pollination gave a full set the species was considered self-sterile. In this way many species which set appreciable amounts of seed on selfing were classified as self-sterile because cross-pollination produced a more complete set. Owing to cross-incompatibility, often only a proportion of the cross-pollinations produced full sets. Sometimes no full sets were obtained. In that case, if the plant looked healthy and the pollen was good it was classified as self-sterile. Where the five plants included both self-fertile and self-sterile individuals the species was regarded as self-sterile. Under this test, species in which the incompatibility reaction merely slowed down self pollen tubes without reducing seed setting would be classified as self-fertile though under open-pollination they might be effectively self-sterile.

The authenticity of the generic name was always checked, and usually the specific name also. Forms which were similar enough to appear to be the same species were counted as single species in the table. The total of 182 species may therefore be an underestimate. The alternatives to this treatment would have involved extensive taxonomic studies, or if every specific name had been accepted, inflated figures which would have given an exaggerated importance to the survey.

Of 182 species distributed over 12 tribes (classification according to O. E. Schulz) 80 were self-sterile and 102 self-fertile. This need not represent the relative frequencies of the two kinds in the entire family, for as self-fertile species are more readily maintained in botanic gardens they might tend to be over-represented. In any case we are not so concerned with overall frequencies as with the distribution among the tribes and genera. Of the eleven tribes sampled, the only one containing no self-sterile species is the Streptantheae, of which only a single species of *Caulanthus* was tested.

Attempts at a phylogenetic system of classification of the Cruciferae have been frustrated by the fact that all characters of possible systematic importance vary independently of one another. However, one must assume that the most recent classification (that of Schulz published in 1936) comes nearest to being a natural one.

That being so, it would appear that all streams of evolution within the family have contained self-sterile species. Indeed we can go further, for of the 17 genera of which four or more species have been tested, only *Lepidium*, *Aethionema*, *Draba* and *Erophila* (four species according to Winge, 1940) presented only self-fertile species. Even more remarkable, only one genus, *Iberis*, presented only self-sterile species. But in most characters which vary within a family one expects a strong correlation between the species of a genus. Here there seems to be little positive correlation, or even a negative one. The formal explanation of this would be that there is some force acting to maintain diversity in respect of self-fertility and self-sterility

even in small taxonomic units like genera. The nature of this force presents a major problem in plant evolution, which cannot be discussed fully here.

It does appear, however, that for the survival and evolution of a taxonomic group, a balance between outbreeding and inbreeding is an advantage. The outbreeding species would provide the store of genetic variation which had been used in the past and would be used in future as the source of new genotypes needed in new environments. The inbreeding species, arising from the outbreeding species as evoked by circumstances, would provide the genetic solutions to the problems of survival presented at the moment by divers environments, but they would continually become obsolete with the passage of time. One might say that the inbreeding species ensure the survival of the group at any instant but the outbreeding species ensure the survival of the group *through* time. One must point out that the inbreeding species are not of course completely self-fertilising, for, as stated earlier, they all have functioning nectaries and will therefore undergo finite, if small, amounts of cross-pollination.

That the Cruciferae present such a clear example of this variation in degree of outbreeding is probably because they have utilised in the main a single outbreeding mechanism—self-incompatibility. A classification of the family into self-fertile and self-sterile species thus gives us directly a classification into outbreeding and inbreeding species.

It may be noted in passing that other authors (*e.g.* Kirchner, 1905, and Beddows, 1931) have observed a strong correlation between the perennial habit and self-sterility. Such a correlation is present in the Cruciferae but it is rather weak. Out of 80 self-sterile species, 34 (42·5 per cent.) are perennials while out of 102 self-fertiles only 26 (25·5 per cent.) are perennials. The Brassiceae, with the highest frequency of self-sterility (22 out of 35), contain only 5 perennials. all self-sterile.

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8. SUMMARY

The demonstration that incompatibility in *Iberis amara* is governed by a sporophytically controlled reaction of both pollen and style leads to a re-examination of published data on other Crucifers.

1. Published data on *Cardamine pratensis*, *Capsella grandiflora* and *Brassica oleracea* can all be shown to be in concordance with the *Iberis results*.

2. Unpublished data on *Raphanus sativus* and *Brassica campestris* are equally consistent.

3. It is concluded that self-incompatibility throughout the Cruciferae is probably due to the same mechanism.

4. This is strengthened by the fact that incompatible pollen is inhibited before the stigma has been penetrated in all Crucifers examined for pollen growth.

5. The continued self-sterility of artificial tetraploids induced in *Brassica* and *Raphanus* is explained by sporophytic control.

A survey of 182 species, of which 80 are self-incompatible, indicates a systematic distribution of self-incompatibility. Tribes and even genera tend to contain both outbreeding and inbreeding species. This indicates that both inbreeding and outbreeding are necessary for the evolution and survival of a tribe or genus.

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INCOMPATIBILITY IN *THEOBROMA CACAO*

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I. INCOMPATIBILITY MECHANISMS

GERSTEL (1950) credits Hughes with first recognising a case of incompatibility governed by multiple alleles exhibiting dominance combined with sporophytic control of the pollen reaction. He states that the hypothesis was given in a thesis on sterility in *Crepis foetida*. In an account of the system, Hughes and Babcock (1950) have pointed out that their results could not be reconciled with any accepted hypothesis of incompatibility.

Three major hypotheses had previously been put forward. In the first, typified by *Nicotiana sanderae*, a multiple series of alleles at a single locus with each allele acting independently and gametophytic control of the pollen reaction govern the mechanism. With such a system four intra-sterile groups are sometimes obtained from a cross but all are inter-fertile and will backcross to either parent. Hughes and Babcock with *Crepis* were able to distinguish among the progeny of a cross four intra-sterile groups but these groups were not fully inter-fertile nor would all of them backcross successfully with their parents. Also obtained in *Crepis* were reciprocal cross differences which are unknown among the *Nicotiana* types except where homozygotes have been obtained by an artificial breakdown of incompatibility.

Riley (1936) assumed sporophytic control of the pollen reaction and two pairs of alleles to explain his results with *Capsella grandiflora*. Hughes and Babcock together with Gerstel (1950), who found a *Crepis* type system in *Parthenium argentatum*, accept Riley's hypothesis, and the only similarities with their own data considered by them are those of sporophytic control of the pollen reaction, dominance of alleles and homomorphic structure of the flower.

A third group of flowering plants, all of which are heteromorphic, have sporophytic control of the pollen reaction and only two alleles at any one locus. Modifying alleles may occur at other loci to make up the complete incompatibility system. Comparatively few compatibility groups may be formed with these factors and reciprocal cross differences do not occur, whereas in *Crepis* seven groups were distinguished among the progeny of a single cross and reciprocal cross differences were found. It was evident to Hughes and Babcock that a new hypothesis would have to be formulated to agree with their experimental findings.

The hypothesis they applied to *Crepis* has been modified by Crowe (1954) to explain results obtained with *Cosmos bipinnatus*. In this

species some of the alleles exhibit dominance in the style, in addition to the pollen, but the dominance sequence is not identical in both style and pollen.

2. PREVIOUS WORK ON *THEOBROMA CACAO*

Incompatibility in cacao has interested research workers wherever the crop has been extensively grown. Over most of these areas the tree is grown from seed but where plant breeding has led to the production of improved selections vegetatively propagated material has begun to replace the seedling trees. As the crop directly depends on successful pollination a knowledge of incompatibility, its cause and incidence, is of major importance.

First experimental results indicating its existence in cacao were published by Pound in 1931. The trees he tested could be divided on their setting capacity into those which were self-compatible and those which were self-incompatible. In the following year he demonstrated that pollen from a self-compatible is effective on any stigma but that pollen from a self-incompatible would only cause setting on a self-compatible. Further reports by Marshall (1933) and Voelcker (1936, 1937) confirmed these earlier findings and illustrated their wide application to the Trinidad population. Cope (1938), however, working with young trees found a range from absolute self-incompatibility to self-compatibility. He was not supported by Ostendorf (1948) who, on the evidence of work at Semarang, found the division between the two classes to be clear cut.

Indications of a change from the self-incompatible to self-compatible state was obtained by Pound (1934) and he suggested that physiological factors could modify the genetic expression of incompatibility at certain seasons of the year. This was termed by Pound conditional self-compatibility. His setting criterion was criticised by Voelcker (1936, 1937) who found that a count of sets after six days may give a false impression of genetic compatibility and that a truer picture is obtained after a fourteen-day interval. Voelcker's own results give no evidence of conditional self-compatibility. Data published by other workers (Cope, 1938; Ostendorf, 1948) show that there is a small seasonal fluctuation in setting capacity, but they do not consider there to be a change from incompatibility to compatibility.

Posnette (1945) on testing a population introduced to Trinidad from the headwaters of the Amazon (Pound, 1938) found all the trees to be self-incompatible but to some extent cross compatible. They would also cross successfully with the self-incompatibles of the cultivated Trinidad population. A further instance of two self-incompatibles being successfully crossed is given by Müntzing (1947) working in Ecuador.

The cytological basis of incompatibility was studied by Cope (1938, 1939). A statistical comparison of pollen tube growth in compatible and incompatible stigmas was insignificant. He traced the tubes into the ovules and found that up to 30 hours after pollination the male and female gametes behaved indentically in the two types of cross. He suggests that the mechanism causing abscission of the flower depends on the degree of nuclear activity within the ovules. In a sterile cross the rate of division of the endosperm nuclei is much less than in a fertile cross and the abscission mechanism comes into operation.

3. MATERIAL AND METHODS

The material used in this study came as seed from Pound's (1938) collection of cacao at Marper Estate, Trinidad. Most of this collection had come from the upper tributaries of the Amazon where the parent trees had been selected for possible resistance to witches' broom disease, *Marasmius perniciosus*. Posnette, in making the introduction to the Gold Coast in 1944, found all the trees to be self-incompatible. The beans introduced to the Gold Coast were planted at Tafo in 1945.

The trees resulting from three pods, designated T.60, T.63 and T.86, were the first to be tested as they represented combinations of three of Pound's selections Na 32, Pa 7 and Pa 35. Thus T.60 was derived from the cross Pa 7 × Na 32 ; T.63 from Pa 35 × Na 32 and T.86 from Pa 35 × Pa 7.

Flowers were protected from insect visitation by gauze-covered tubes. Emasculation is unnecessary in cacao. A minimum of five but mainly ten pollinations were made for each cross and were considered successful if the flowers remained on the tree for ten days. The incompatibility reaction is decisive and most of the flowers of an incompatible cross had fallen by the fourth or fifth day.

The 28 trees comprising T.60 were selfed and as many as possible crosses between sister plants were made. T.63 and T.86, each numbering 27 trees, were similarly tested.

TABLE 1

	♂	A	I	B	F	Na 32	E	J	D	C	G	H
♀	S genes	1·2 1·4	1·3 1·5	2·5	2·5	2·4	2·3	3·5	3·4	4·5	4·5	5·5
A	1·2 1·4	−	−	+	...	+	+	+	+	+	+	+
I	1·3 1·5	−	−	...	+	+	...	+	...	...	...	+
B	2·5	+	+	−	...	−	−	+	+	+	...	+
F	2·5	+	+	−	−	−	−	+	+	...	+	+
Na 32	2·4	...	...	...	...	−	...	...	...	...	...	...
E	2·3	+	+	−	−	−	−	−	−	+	+	+
J	3·5	...	+	...	+	+	...	−	−	...	...	+
D	3·4	+	+	+	+	+	−	−	−	+	...	+
C	4·5	+	+	+	+	+	+	+	...	−	−	+
G	4·5	+	+	+	+	+	+	+	+	−	−	+
H	5·5	+	...	...	+	+	...	...	...	...	...	−

4. RESULTS

It was found that the 28 plants of T.60 could be separated into three groups (A, B, C) according to their cross relationships. The three groups were intra-sterile and inter-fertile and differed in the number of plants they contained. Thus group A contained 16 trees, group B, 7 trees and group C, 5 trees. The plants of T.86 were likewise

separated into three intra-sterile and inter-fertile groups, H, I and J consisting of 9, 13 and 5 trees respectively.

Among the T.63 trees four groups were found (D, E, F, G) two of which, E and F, were cross-sterile and could only be distinguished by their relation to the group D. Group E was cross-sterile and group F was cross-fertile with group D. The fourth group, G, was completely intra-sterile and inter-fertile. The number of trees in D, E, F and G was 6, 6, 6 and 9 respectively.

On cross pollinating between the ten groups recognised in T.60, T.63 and T.86 it was found that several were cross-sterile. We have only been able to backcross to Na 32 as the parental clones Na 32, Pa 7 and Pa 35 were not introduced to the Gold Coast until October 1951. The clone Na 32 bore sufficient flowers in 1953 to carry out backcrossing. The results of crossing the ten groups and the backcross are given in table 1.

5. GENETIC HYPOTHESIS

The following genetic hypothesis is proposed to explain the results :—

1. That success or failure of a cross depends on the diploid constitution of both male and female parent.

2. That in the clones Pa 7, Pa 35 and Na 32 five alleles at a single locus are operative.

3. These alleles differ in dominance but two are equal. They follow the sequence :—

$$S.1 > 2 = 3 > 4 > 5.$$

The sterility gene was previously designated W (Knight and Rogers, 1953).

4. Dominance according to the sequence is exhibited by both male and female organs.

Assuming the clones Pa 7, Pa 35 and Na 32 to be of the constitution S1.5, S3.5 and S2.4 respectively we have as the cross producing the T.60 trees :—

$$S1.5 \times S2.4.$$

The offspring of this cross will be :—

$$S1.2 \quad S1.4 \quad S2.5 \quad S4.5$$

but because S1 is dominant to S2 and S4 in the two groups S1.2 and S1.4 these groups will be indistinguishable in their cross relations. Only three intra-sterile inter-fertile groups were found in the analysis of T.60 with group A containing twice, within experimental variation, as many trees as either of the other groups.

Applying the premises of the hypothesis to T.86 the parents are genetically S3.5 and S1.5 and will give rise to :—

$$S1.3 \quad S1.5 \quad S3.5 \quad S5.5$$

S1 being dominant to S3 and S5, the two groups S1.3 and S1.5 will

behave identically in a cross and cannot be separated by test pollinations. As with T.60 only 3 intra-sterile inter-fertile groups were found in T.86 with one of the groups (I) larger than the other two.

T.63 was the outcome of the cross between Pa 35 and Na 32. The genes involved according to the hypothesis will be S3.5 and S2.4 giving rise to :—

S3.4 S2.3 S2.5 S4.5

The groups S2.3 and S2.5 could be distinguished experimentally when crossed with S3.4. If this test cross was made S2.3 would be incompatible with S3.4, having the gene S3 in common and equal in action to S2, but S2.5 would be compatible having no common gene. S4.5 would make a fourth distinct group. Four groups were found experimentally in T.63 and agree in their cross relations with those deduced from the hypothesis.

Dominance in the ovary in most cases prevents the separation of the progeny of a cross into the expected four groups. Only when two alleles of equal dominance are associated with weaker alleles, as in T.63, are four groups distinguishable.

When the compatibility between the groups of T.60 and T.86 is considered it is evident from the hypothesis that the largest group in T.60 should not intercross successfully with the largest groups in T.86 but that all the other groups of these two T. numbers should be fully inter-fertile. With the crossing of the groups of T.63 and T.60 it would be expected that S2.3 and S2.5 of T.63 would fail with S2.5 of T.60 and the S4.5 of both would be incompatible. Lastly, crossing between the groups of T.86 and T.63, S3.5 of the former would fail with S3.4 and S2.3 of the latter. All other groups would be fully compatible. On backcrossing to S2.4 (Na 32) failure would be obtained with S2.5 of T.60 and S2.3 and S2.5 of T.63. The results obtained in the field agree in detail with those deduced from the hypothesis.

6. CYTOLOGICAL INVESTIGATION

A cytological investigation was carried out to verify previous findings on cacao. Pollen tube growth in known compatible and incompatible crosses was compared at set intervals from the time of pollination. Ovaries were fixed in chrom-acetic formalin, cleared in *n*-butyl alcohol and embedded in paraffin wax. They were then sectioned at 12 μ and stained in crystal violet following the modified schedule of Smith (1934). The pollen tube studies showed that the rate of pollen tube growth was the same in both sterile and fertile crosses. Tubes could be traced down the stylar canal to the ovules.

Sections through the ovary were identical in both types of cross up to 36 hours after pollination. In sterile and fertile crosses the ovules show a breakdown of one of the synergids and the presence of three endosperm nucleoli. These features are particularly noticeable thirty hours after pollination. In an unpollinated ovary the synergids

remain intact and there are only two endosperm nucleoli. In both types of cross the male gamete that fuses with the endosperm was easily distinguished within the ovule but the second male gamete, that in other species fuses with the egg, was not detected. Our sections only differed from those of the variety, Trinitario, depicted by Cope (1939), in that we were unable to detect the second male gamete and its fusion with the egg. Since the zygote does not normally divide until at least forty-five days after fertilisation (Cheeseman, 1927), by which time an incompatibly pollinated flower has fallen, fusion of male and egg nucleus in an incompatible cross cannot be assumed. From thirty-six hours until the flower of a sterile cross fell at about four days there was a progressive degeneration in the cytoplasm of the ovary. The ovular nuclei become increasingly difficult to detect.

There can be little doubt that the contents of the pollen tubes are liberated in the ovules of cacao and that failure to set results from a subsequent incompatibility.

7. DISCUSSION

Multiple allelic incompatibility must depend for its operation on as many specific reactions as there are distinguishable incompatible groups. More than forty have been detected in some species and it has been suggested that the reaction depends for its specificity on complex polysaccharides or proteins. Analogies have been drawn with the serological antigen-antibody reaction and evidence in its support has been obtained by Lewis (1952). Since the inhibitory reaction of the style, according to Lewis, is preformed and not developed as a result of an incompatible stimulus, a further analogy has been drawn with human blood groups where the AB antigens are naturally occurring.

The antibodies in the serum of one blood group oppose antigens other than the one borne by that group—B group serum contains anti-A antibody—whereas, in the plant's incompatibility reaction, genetically similar groups are opposed and the active substances must be spatially separated. In cacao there is no inhibition in the style and it is suggested that these substances are confined to the cytoplasm of the ovules and pollen. This may be a more primitive condition since it leads, as will be discussed later, to the loss of ovules. It is probable that the incompatibility substances are formed prior to gametogenesis, since they are determined by the diploid constitution of the parent, and this principle may hold for all cases where incompatibility is sporophytically determined.

Autopolyploidy is unlikely to affect the production of " antigens " in pollen when the reaction is sporophytic and the genes exhibit dominance. Tetraploid plants of *Parthenium* showed the same reactions as their diploid sibs (Gerstel, 1950). There is no indication in our data of competitive interaction or mutual weakening of genes. All the combinations of S1 with S2, S3, S4, S5 behave identically. Whether

this absolute dominance would be manifest in a triallelic tetraploid, such as S1223, is of interest in the consideration of " antibody " or " antigen " formation. It might indicate if the " antigen " produced is solely that corresponding to the dominant gene or is at so high a concentration, in diploids, as to mask the " antigen " of the lesser gene. It is known from T.63 that two alleles of equal dominance occur in male and female organs, corresponding with S2 and S3, without a reduction in the strength of either.

Lewis (1947) found from his work with tetraploid *Oenothera* that dominance could occur between the genes of diploid pollen but that the genes of the style retained their independent action. He points out that this independence is of natural selective value. In a diploid, where the pollen reaction is gametophytic, dominance in the style permits self-compatibility and inbreeding. If the reaction is sporophytic, as it is in cacao, dominance in both style and pollen does not lead to self-compatibility and in this case independence of the stylar genes is no longer of natural selective importance. Dominance in the style results in a higher compatibility between sibs and prevents the reciprocal cross differences found in *Parthenium* and *Crepis* where the genes are independent. *Cosmos* is apparently intermediate between *Crepis* and cacao, having some alleles independent in the style that are dominant in the pollen. It has been pointed out (Crowe, 1954) that there is some evidence for dominance between the alleles of the style in *Parthenium*, although this possibility is not mentioned by Gerstel. The raising of compatibility between sibs of cacao lowers the outbreeding efficiency (Bateman, 1953) but conserves generative material where the cacao population is restricted, as it frequently is, in the plant's natural habit.

To achieve incompatibility in cacao there is an enormous loss of flowers which is compensated to some extent by their copious production by self-incompatible as opposed to self-compatible trees. Self-incompatibility does not greatly reduce the production of fruit in Trinitario (Cope, 1938). In other plants the stylar sieve segregates incompatible pollen and leaves the ovules open to future fertilisation. Other instances in the literature of failure subsequent to the pollen tubes reaching the ovules are *Gasteria verrucosa* (Sears, 1937) and *Hemerocallis citrina* (Stout and Chandler, 1933). Both are rhizomatous and have many ovules within the ovary. The effect on reproduction of loss of ovules may be lessened by vegetative propagation or, when a pollination is mixed, sufficient ovules may be compatibly pollinated for the fruit to continue development.

An hypothesis similar to the one adopted for cacao could explain the results with *Capsella grandiflora* (Riley, 1936). A system similar to that in cacao was depicted by Bateman (1952) when he considered from first principles the combination of known incompatibility factors. Genetically cacao resembles Bateman's scheme J. He states that this scheme has a close empirical resemblance to the results obtained in

Capsella. If this is the true explanation multiple allelic incompatibility together with sporophytic control of the pollen reaction will have been found in the three phylogenetically separated families Sterculiaceae, Compositae and Cruciferae.

The cacao population considered in this paper contains no self-compatible trees. In a second paper it is hoped to relate this system with that evidently operating in Trinitario, where a proportion of the trees are fully self-compatible, and to discuss the evolution of the two systems.

8. SUMMARY

1. Incompatibility among the progeny of three clones of Amazon cacao crossed in all combinations was studied.

2. All the progeny were found to be self-sterile but to a limited extent cross-fertile. On the basis of cross relationships each of the progenies of two crosses was separated into three intra-sterile but inter-fertile groups. A third cross had one distinct intra-sterile inter-fertile group and three intra-sterile groups that were not fully inter-fertile.

3. A genetic hypothesis was proposed to explain the results. It is based on five alleles that exhibit dominance according to the sequence $S.1 > 2 = 3 > 4 > 5$. The genes occur at the same locus.

4. A cytological investigation showed that incompatibility occurs after the pollen tubes have penetrated the ovules. In the cacao types considered incompatibility is not achieved without a heavy loss of generative material.

5. The incompatibility reaction of pollen and ovule is sporophytic : it is determined by the diploid constitution of the male and female parent respectively.

6. The incompatibility system in Amazon cacao is discussed in relation to dominant gene action and the " antigen-antibody " theory of incompatibility. It is suggested that the active incompatibility substances are confined to the cytoplasm of ovules and pollen.

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THE LIFE HISTORY AND GENETIC SYSTEM OF *LEUCOPOGON JUNIPERINUS*

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1. INTRODUCTION

Leucopogon juniperinus R. Br. has a wide but apparently discontinuous distribution in the coastal and tableland regions of eastern Australia. It extends over a latitudinal range of nearly 1000 miles, from Gympie (Queensland) to Gippsland, and is found at altitudes from sea level to 4000 feet. Throughout this range, the species shows a high degree of uniformity in morphological characters, and it is apparent that this uniformity has a cytological basis.

Material has been examined from the following localities :—

Queensland : Mt. Coot-tha (4 plants).

N.S.W. : Chatswood (3 plants), East Gordon (11), West Pymble (4), Lane Cove (7), Yagoona (3), Newport (2), Yadboro Creek (4), Milton (1), Wandanian (3), Wauchope (2), Tooloom (2).

Aceto-lacmoid squashes were used for the examination of mitosis in leaf and ovule tissue, and for meiosis in pollen mother cells. Dissection squash methods were used to supplement section techniques in the study of embryo-sac development, and proved more valuable for the determination of chromosome constitution in embryo sacs, embryos and endosperms. Pollen tube development in styles was examined in longitudinal sections cut on a freezing microtome at *ca.* 100 microns, and stained either in iodine or in acid fuchsin. Pollen size measurements were made by the use of a planimeter on camera lucida drawings.

2. OBSERVATIONS

(1) *Chromosome constitution*

In the 46 plants examined, and in 22 embryos dissected from young seeds, the somatic chromosome number was twelve. The chromosomes are of approximately equal length, and in size are comparable with those of the 4-series diploid species *L. setiger*, *L. esquamatus* and *L. propinquus*, rather than with the chromosomes of the 6-series diploid species *L. ericoides* and *L. microphyllus* (fig. 8C).

Following mild cold treatment (4-6° C. for 48 hours) an apparent banding of the chromosomes can be seen in late prophase (fig. 8A, B), suggesting the existence of heterochromatic segments in all chromosomes. It is also possible to identify two short chromosomes in somatic tissue. Similar stages in cold-treated endosperms show three short chromosomes, which must therefore be homologues of the pairing chromosome sets, and not individuals of the univalent set.

Meiosis in P.M.Cs. has been described previously (Smith-White, 1948*b*). At 1-M, four bivalents and four univalents are regularly present. There is never any indication of even temporary association

in prophase between the univalents, either by chiasmata or secondary attraction. The mechanism suggested by Catcheside (1950) to account for the polarised segregation of B chromosomes in *Parthenium* could not be operative in the present case.

(2) *The development of the pollen*

Pollen development after meiosis conforms with that usual in the *Styphelieae* (Smith-White, 1948*a*). A migration of the microspore nuclei (fig. 1A) results in initially unequal microspores (fig. 1B, C). The small microspores survive for a time, and contribute to the deposition of cell wall (fig. 1D), but probably never undergo any mitosis. They contain degenerate cytoplasm when the functional microspore is still uninucleate (fig. 1E). The large microspore increases considerably in size, and becomes a binucleate pollen grain (fig. 1F, G). Mature pollen is apparently single but strictly monad in nature.

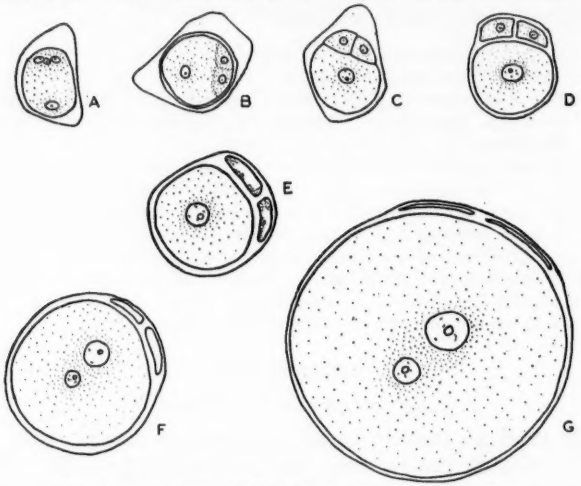


FIG. 1A-G. ×640. Pollen development in *L. juniperinus*.

A. Pollen mother cell following meiosis and nuclear migration.
B, C. Early stages in the development of the tetrad.
D, E. Uninucleate stages in pollen development.
F, A. A young binucleate pollen grain. The smaller nucleus, assumed to be the generative nucleus, is distal to the aborted cells.
G. The mature pollen grain.

This type of pollen development is found in most genera and species of the *Styphelieae*. It is characteristic of the tribe, and therefore antecedent to the condition of triploidy in *L. juniperinus*. A rather similar type of pollen development has been described in the Cyperaceae (Juel, 1900; Stout, 1912; Piech, 1928; Tanaka, 1940, 1941).

In the young pollen grain, the two nuclei are unequal in size and are asymmetrically placed. The smaller nucleus usually lies distally

or obliquely to the aborted cells. If it may be assumed that the smaller nucleus is the generative one, this orientation does not conform to that characteristic of angiosperm pollen, as demonstrated by Darlington and Mather (1949) in *Hyacinthus* and by de Almeida and Sampaya (1950) in *Luzula*. Samuelsson (1913, *cf.* Maheshwari, 1950) reported that in *Vaccinium* the pollen tetrads produced their generative cells near the peripheral walls, but in tetrad pollen in *Richea* (fig. 3A, B) and in *Epacris*, the orientation of the generative nuclei is similar to that of *Luzula*.

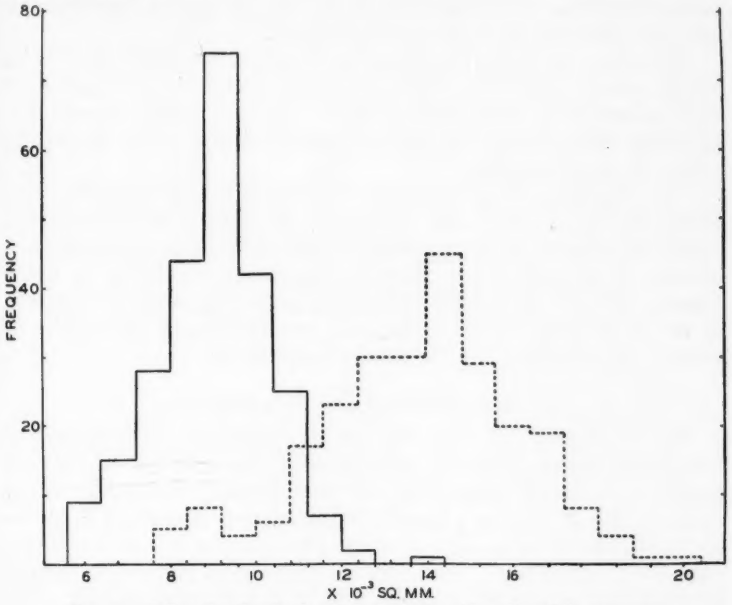


FIG. 2.—Pollen size distribution in *L. juniperinus* (dotted) and *L. esquamatus*.

The granular cytoplasm of the young pollen grain is densely packed with plastids and starch, and a direct determination of chromosome constitution in the P.G. mitosis has not been achieved. An analysis of variation in pollen size in comparison with that of the related diploid species *L. esquamatus* ($2n = 8$) was made in an attempt to infer cytological heterogeneity (fig. 2). The pollen of *L. juniperinus* has a larger mean size, and a significantly higher standard deviation. However, both size distributions appear to be unimodal, and neither shows any significant deviation from normality. In *L. esquamatus*, the whole of the variability must be dependent upon differences in food storage, and in *L. juniperinus*, a similar explanation is probable. The data do not deny the view that the functional pollen is of uniform constitution.

The extent of pollen fertility has been described previously (loc. cit.) where it was shown to be higher than would be expected with random univalent segregation. Culture of pollen in 25-30 per cent. sucrose solution, using La Cour's cellophane square technique (Darlington and La Cour, 1948) results in rather erratic germination after 48 hours. No approach to 100 per cent. germination of apparently sound pollen has been achieved, but there does not appear to be any correlation between germination and pollen size. It is unlikely that germination of all potentially functional pollen can be obtained in sucrose solution. The tube cytoplasm, like the pollen cytoplasm, is granular and contains a great deal of starch.

Following artificial self-pollination of excised flowers in 2in. × 1 in. phials, pollen germination and pollen tube penetration of styles have been observed in twenty-four hours. On the plant, flowers are markedly protandrous, and self-pollination seems to occur frequently before the corolla opens.

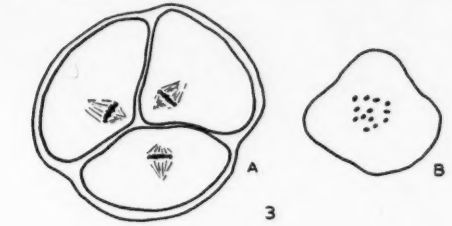
The *Styphelieae* are particularly suited for the study of pollen tube growth in styles. As in the Ericaceae, the style is hollow, and the pollen tubes grow down a central canal. The heavy concentration of starch in the tubes gives them an appearance not shared by stylar tissues. Using section-crush techniques, pollen tubes have been seen in the lower parts of the styles, and penetrating the micropyles of ovules. It is unlikely that the species is apomictic.

(3) *Megasporogenesis and the embryo sac*

Brough (1924) has described the morphological development of the female gametophyte in *Styphelia longifolia*, and his studies will provide a basis for comparison in the following treatment. In *S. longifolia*, the monosporic, 8-nucleate embryo sac is derived from the micropylar megaspore. The other three megaspores do not degenerate until after fertilisation. The primary endosperm nucleus is formed by the fusion of two polar nuclei, and is consequently diploid. The addition of a haploid male nucleus gives a triploid endosperm. Brough's treatment was entirely morphological, but triploid endosperms associated with diploid embryos ($2x = 8$, $3x = 12$) have been observed in *S. longifolia*, *S. triflora*, *S. laeta*, *S. tubiflora*, *Leucopogon esquamatus*, *L. rotundifolius*, *L. propinquus* and *L. setiger* (fig. 8E, F). These determinations confirm Brough's interpretation.

The pistil of *L. juniperinus* is composed of five fused carpels. It is differentiated into a 5-loculed ovary, a hollow style 7-10 mm. long, and a small glandular stigma. In each locule there is a single anatropous ovule on an apical placenta. In the young ovule a single massive integument surrounds a small nucellus comprising only a single axial row of cells and a nucellar epidermis. The terminal cell of the axial row becomes the E.S.M.C. At the time of meiosis the nucellus is deeply embedded in the integument, and is connected to the external micropyle by a long narrow canal.

Meiosis in the E.S.M.C.—The course of meiosis in the E.S.M.C. appears to be identical with that already described for the P.M.C. In late prophase and at first metaphase four bivalents and four univalents are present. The univalents lie off the plate, towards either or both poles (fig. 4A, B), and may be pushed almost to the extremities of the spindle. The observed univalent segregation (table 1) seems to be compatible with polarisation, and less so with random segregation.



3

FIGS. 3 and 4. ×2080.

FIG. 3A, B.—The pollen grain mitoses in tetrad pollen of *Richea dracophylla* (Epacridaceae, tribe *Epacrideae*). 3A eccentric spindles in the three pollen grains of the tetrad. 3B Polar View of the metaphase plate in the fourth pollen grain of the same tetrad.

FIG. 4A, B.—First metaphase in the E.S.M.C. in *L. juniperinus*. 4A A 2/2 segregation of the univalents. 4B A 0/4 segregation favouring the micropylar pole.

Metaphase counts of the second division have not been obtained, but the divisions, seen in side view, are apparently normal, and a row of four megaspores is produced. The megaspores are at first of approximately equal size. There is no parallel to the nuclear migration found in the P.M.C.

TABLE 1

Univalent distribution at 1-M in E.S.M.Cs. L. juniperinus

Segregation . .	Micropylar/ Chalazal	0/4	1/3	2/2	3/1	4/0
Observed mother cells .	...	1	...	1	3 *	2

* In one of these cells, the distribution was actually 3/0, with the fourth univalent lagging on the plate.

The development of the embryo sac.—The functional E.S. is always developed from the micropylar megaspore. It undergoes three mitoses to become 8-nucleate, and following the fusion of the polar nuclei, 7-nucleate. It contains an egg apparatus of two large terminally

striate synergids and a large egg nucleus. There is a single endosperm nucleus and three small degenerate antipodal cells (fig. 5A-E). The three non-functional megaspores remain large and conspicuous even after fertilisation. Except for this feature, and for the selection of the micropylar megaspore, E.S. development conforms to the " normal" angiosperm type. Even where the micropylar megaspore fails, the others show no trace of development. There is no megaspore competition of the *Oenothera muricata* type.

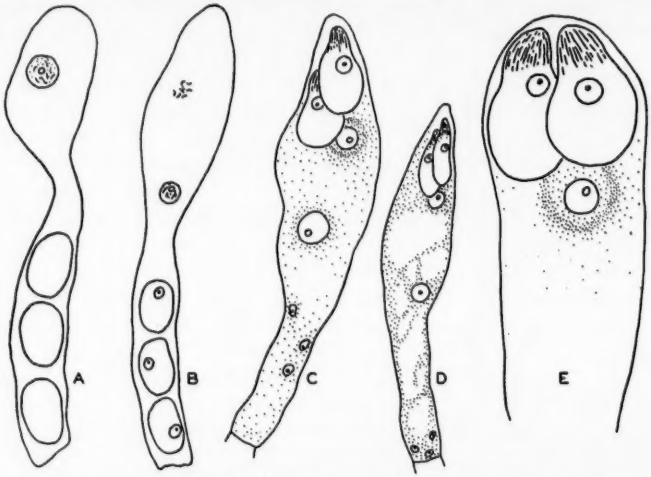


FIG. 5A-E. ×640. Stages in embryo sac development in *L. juniperinus*.

A. The uninucleate embryo sac, with the three non-functional megaspores below. Lacmoid crush.

B. The second embryo sac division. Lower nucleus in late prophase, upper nucleus in metaphase, with eight chromosomes. Lacmoid crush.

C. The seven-nucleate embryo sac from a nearly mature ovule. The striate synergids are conspicuous, the other nuclei less so, and the sac is not obviously vacuolate. Dissection-Lacmoid.

D. A similar stage to 5C. Paraffin section-Gentian Violet.

E. A fully mature embryo sac. Lacmoid-crush. The size of the sac has probably been influenced by flattening. The synergids are conspicuous.

Embryo sac abortion.—When all five embryo sacs in a nearly mature flower are examined, they rarely show comparable development (fig. 6). One, two, or more rarely three, may show " full " development, as judged by the presence of conspicuous synergids, and the others are underdeveloped. Observation of all five embryo sacs in a flower has been achieved in five cases (table 2). Pre-pollination E.S. abortion approximates to 75 per cent., and this frequency should be compared with data on seed fertility described below.

(4) *The development of the seed*

Pollination is necessary for fruit development, which has failed after emasculation of flowers on plants transferred to the garden.

Stages in the development of the embryo and endosperm are illustrated in fig. 7A-C. The persistent endosperm is cellular. The

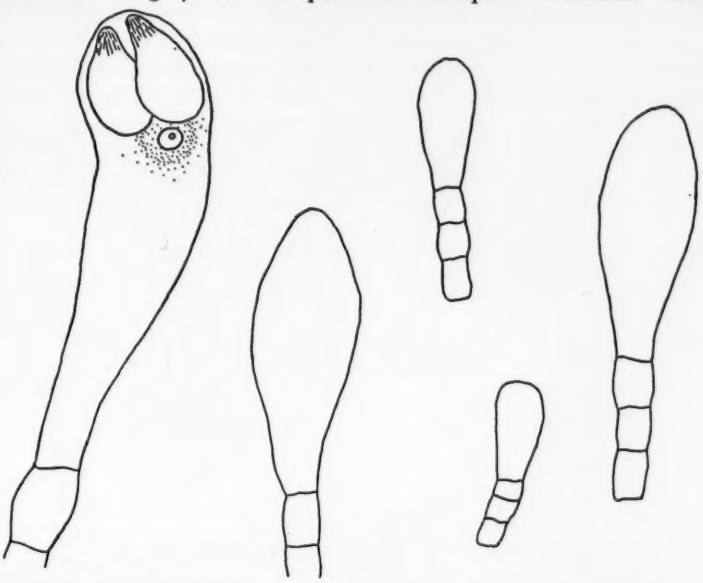


FIG. 6.—Outlines of the embryo sacs from the five ovules of a nearly mature flower bud. Only one is functional. In the other four the synergids are not developed, and they appear to have failed after partial growth. ×640.

obsolete embryo sac can be recognised in stages later than that shown in fig. 7C. In that example two degenerate nuclei are shown in the

TABLE 2

Embryo sac fertility in L. juniperinus *from East Gordon*

	Functional E.Ss. per ovary (4 ovules)					
	0	1	2	3	4	5
Number of E.Ss. observed per ovary: 5	1	3	1	...	...	...
4	3	1	3	1	...	...
3	1	...	2	...	...	...
2	...	4	...	...	...	...
Totals . . .	5	8	6	1	...	...
Expectation on basis $(\frac{3}{4}+\frac{1}{4})^5$	4·75	7·91	5·27	1·76	0·29	0·02

Embryo sac fertility :

(*a*) On assumption that E.Ss. not observed include functional and aborted in the same proportion . . . . . 31·1 per cent.

(*b*) On assumption that embryo sacs not observed were undeveloped 23·0 per cent.

Fit to $(\frac{3}{4}+\frac{1}{4})^5$ distribution, $\chi^2 = 0{\cdot}443$. 3 D.F. $P = 0{\cdot}92$.

lower half of the sac, and are probably two of the three antipodals; the degenerate non-functional megaspores can still be recognised. The development is of the "caryophyllad" type as defined by Johansen (1950), and has a remarkable similarity to that of *Medicago lupulina*. A greatly elongated suspensor is apparently characteristic of the *Styphelieae*.

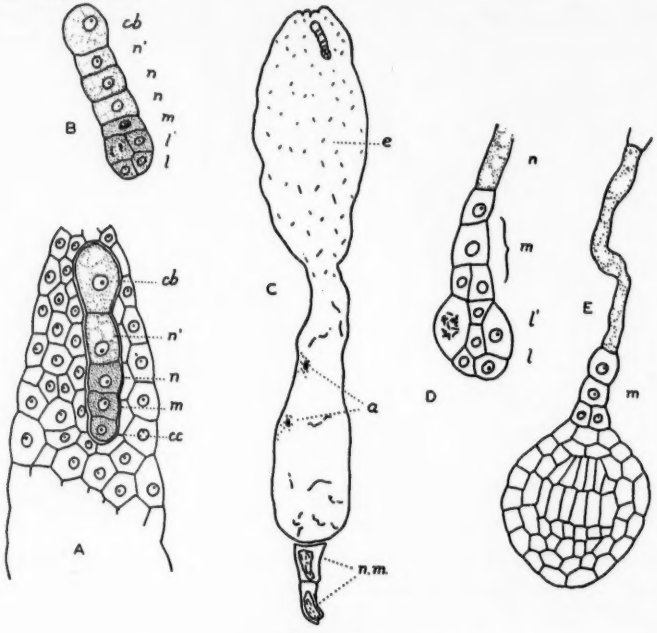


FIG. 7A-E.
Stages in embryo development in *L. juniperinus* (notation following Johansen, 1950).

A. A five-celled embryo. *cb* and *n'* are differentiating, and possess vacuolate lightly staining cytoplasm. The whole of the embryo proper is derived from the cell *cc*. ×640.

B. An embryo with a terminal octet (*l*-*l'*). These and cell *m* appear to be the only ones which have not differentiated. ×640.

C. The same embryo as fig. 7B drawn at a lower magnification. The cellular endosperm (*e*) fills the upper half of the obsolete embryo sac. In the lower half two degenerate antipodal cells (*a*) can be seen. The remnants of the nonfunctional megaspores (*nm*) still persist. ×160.

D, E. Later stages in the development of the embryo. The elongate cell of the suspensor is derived from *n*. ×640.

Mitoses have been observed in embryos ranging in development from that illustrated in fig. 7A to that shown in fig. 7D. The chromosome complement is invariably 12. Zygotic elimination of cytologically unbalanced embryos is not a factor of any importance in the stability of the species.

The endosperm is invariably pentaploid ($5x = 20$). Counts have been obtained on 36 separate endosperms, including nine from which

embryo counts were also obtained. Regular pentaploidy of the endosperm, associated with equally regular triploidy of the embryos, verifies the previous occurrence of double fertilisation, and is decisive in regard to the chromosome constitutions of the functional pollen grains and embryo sacs. The inference that only 4-chromosome pollen and 8-chromosome embryo sacs function is obligatory.

FIG. 8A-F. ×2080.

The somatic chromosomes of *L. juniperinus* (A-D) and *L. rotundifolius* (E, F).

A. Late prophase in an embryo, following cold treatment $3x = 12$.
B. Late prophase in endosperm, following cold treatment $5x = 20$.
C. Metaphase in embryo $3x = 12$.
D. Metaphase in endosperm $5x = 20$.
E. Metaphase in embryo $2x = 8$.
F. Metaphase in endosperm $3x = 12$.

Seed fertility.—*L. juniperinus* sets fruit abundantly. The proportion of fruit set, and seed fertility, can be estimated in a novel manner. Every ovary has five ovules, but fruits usually contain only one or two seeds. Data on number of seeds per fruit for *L. juniperinus* and other species are given in table 3. In the case of the East Gordon plants, the satisfactory fit of the observed distribution to the binomial $(\frac{3}{4}+\frac{1}{4})^5$ indicates that only about 25 per cent. of ovules develop into seeds, that one functional seed serves to ensure fruit development, and that the fate of any ovule is independent of the fates of the other ovules in the same ovary. Further, it may be estimated that 25 per cent. of flowers fail owing to the absence of effective ovules.

In the Milton material, ovule fertility is much higher, exceeding 40 per cent., and approaches that found for the diploid species *Styphelia tubiflora*. The tetraploid *L. biflorus* has a lower fertility, approximately $12\frac{1}{2}$ per cent. In all these examples, the observed distributions show a satisfactory fit with binomials. The data for *L. setiger* indicate a different basis of seed fertility, involving probably severe competition within the ovary.

The data on pre-pollination embryo sac abortion were obtained from East Gordon plants. It is apparent that E.S. abortion is primarily responsible for seed failure, and this failure can in fact be recognised very early in the development of the fruit. With the evidence for polarised univalent segregation in meiosis and the requirement of an 8-chromosome embryo sac, it may reasonably be concluded that in 25 per cent. of the E.S.M.Cs. there is a full polarisation of the four univalents to the micropylar megaspore. Random segregation would give such an occurrence with a frequency of only $6\frac{1}{4}$ per cent.

TABLE 3

Number of seeds per fruit in L. juniperinus *and in related species*

Species	Locality	Fruits with seeds/fruit						Total fruits	χ^2	P
		0	1	2	3	4	5			
L. juniperinus . .	East Gordon	...	183	126	29	7	0	345	3·40	0
Binomial $(\cdot75+\cdot25)^5$.		*107·34*	*178·91*	*119·27*	*39·76*	*6·63*	*0·44*			
L. juniperinus . .	Milton	...	177	281	203	94	8	763	4·64	0
Binomial $(\cdot57+\cdot43)^5$.		*48·85*	*184·25*	*278·00*	*209·75*	*79·10*	*11·93*			
L. biflorus . . .	Kosciusko	...	150	45	5	0	0	200	0·48	0
Binomial $(\cdot875+\cdot125)^5$		*210·60*	*150·43*	*42·98*	*6·14*	*0·44*	*0·01*			
S. tubiflora . . .	Lane Cove	...	48	78	65	45	12	248	7·08	0
Binomial $(\cdot5+\cdot5)^5$.		*8*	*40*	*80*	*80*	*40*	*8*			
L. setiger . . .	Lane Cove	...	116	8	0	0	0	124		

Note.—In calculation of χ^2, classes with an expected frequency below 5 have been grouped with the next higher class.

In the P.M.C., where " ends " cannot be distinguished, polarisation results in only about 50 per cent. of viable monad pollen. The four univalents must pass to the same 1-A pole in 50 per cent. of cases, or twice the frequency estimated for the E.S.M.Cs. If, however, in the E.S.M.C., polarisation is independent of the micropylar-chalazal axis, it could be inferred that in an additional 25 per cent. of E.S.M.Cs. all univalents would pass to the chalazal pole. The conditions of polarised segregation are probably similar in pollen mother cells and embryo sac mother cells.

3. DISCUSSION

Leucopogon juniperinus is a triploid, in which one haploid chromosome set shows no tendency to pair in meiosis. It must be allotriploid, with a diploid set from one parent and a haploid set from another. The morphological and cytological uniformity of the species, its wide and probably discontinuous range, and the absence of any obvious parental species, suggest that it has had a single and rather remote

origin. It must have a rigid genetic system giving it little evolutionary potential.

In *L. juniperinus* neither apomixis nor zygotic selection can be operative. There is a system of complementary gametic elimination reminiscent of the inheritance of the *rigens* and *curvans* complexes in *Oenothera muricata*. Its genetic system extends that known in *Rosa canina* (Tackholm, 1922, Hurst, 1931). In both, the univalents are carried, apomictically as it were, in the maternal line only. In *L. juniperinus*, however, there is a controlled elimination of univalents in the pollen, and this condition is absent in *R. canina*. The origin of controlled univalent elimination in the pollen was probably dependent upon the prior existence of the monad type of pollen development, and on cytoplasmic polarity. It must be inferred also that in the E.S.M.C. the conditions for polarised segregation were antecedent to the origin of triploidy. Darlington's view (1937, p. 464) that in *R. canina* the control of univalent segregation in the E.S.M.C. was achieved by adaptation in the course of sexual reproduction cannot hold in the present case.

The systems of differentiation.—There are evidently several distinct sets of conditions operating in the development of the embryo sac in *L. juniperinus*, and these may be related to more normal conditions in the angiospermic ovule.

(1) There is a tissue gradient extending over the ovule, which determines the development of the micropylar megaspore. A similar gradient, more often favouring the chalazal megaspore, is normal in angiosperm ovules generally. There is no comparable condition in the anther.

(2) There is an intracellular gradient in the E.S.M.C., and also in the P.M.C., which controls univalent segregation. In the E.S.M.C., this gradient may act in either a micropylar or chalazal direction, and a functional E.S. is formed only when the two gradients act together. The tissue gradient is the stronger, since micropylar megaspores of unsatisfactory constitution show partial development, but chalazal ones never do so, even in the absence of effective micropylar competitors. This condition contrasts with that found in *Oenothera muricata*.

The control of P.M.C. and E.S.M.C. segregation must be similar, and in the former a source of polarity may be suggested in precocity of the normal polarity of the PG mitosis. If a similar gradient is normally present in the angiosperm embryo sac, it follows that the basic causes of differentiation in the pollen grain and embryo sac are in part related.

(3) An undefined set of conditions, different in anther and ovule, determines the development of the sporogenous tissue into microspores or megaspores, pollen grains or embryo sacs. The observations of Nemec, de Mol, Stow, and Naithani (*cf.* Maheshwari, 1950) on the development of embryo-sac like pollen in *Hyacinthus* show that these conditions are basically independent of the gradients just defined.

4. SUMMARY

1. *Leucopogon juniperinus* is a permanent triploid species with a somatic chromosome number of 12. It has a wide distribution and is morphologically and cytologically uniform.

2. The species has retained a sexual system, and its stability as a permanent hybrid is maintained by complementary gametic elimination. Functional pollen is "haploid", and the univalents are excluded from paternal transmission. Functional embryo sacs are "diploid", and carry the univalents. There is a constant relationship of pentaploid endosperm with triploid embryos.

3. Conditions of meiosis are similar in the P.M.C. and E.S.M.C. In both, a similar intracellular polarity must control univalent segregation.

4. Pollen is of the monad type generally characteristic of the tribe *Styphelieae*. The conditions which determine monad development probably also control univalent segregation, and are certainly antecedent to the origin of triploidy.

5. In the embryo sac three conditions can be recognised as being involved in development and differentiation. The first is a *tissue gradient* in the ovule, which is not found in the anther. Second is the *intracellular gradient* which determines univalent segregation. Third is a set of conditions determining femaleness in development.

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GENOTYPIC CONTROL OF CHROMOSOME BEHAVIOUR IN RYE

I. INBRED LINES

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1. INTRODUCTION

GENES within the nucleus are relatively unchanging, permanent hereditary units : they comprise the nuclear genotype. The chromosomes which bear them, on the other hand, change considerably in behaviour and appearance. In this respect the nucleus presents a phenotypic aspect. This nuclear phenotype changes not only during cell division : throughout the life history of any organism it is subject to the control and interaction of the genotype and the environment.

Few attempts have been made to study the genetics of chromosome behaviour. There are exceptions, notably the works of Darlington (1932) ; Beadle (1933) on maize ; Peto (1933) on *Lolium-Festuca* hybrids ; Maeda (1937), Emsweller and Jones (1945) on *Allium* species crosses ; Müntzing and Akdik (1948) and Prakken and Müntzing (1942) on rye.

The problem of genotypic control can be tackled in five ways, (1) by the study of hybrids, and in particular their derivatives (as in *Lolium-Festuca* and *Allium* above), (2) by investigating inbred lines produced from an organism that normally outbreeds (as in rye), (3) by practising selection for a chromosome character (which has not yet been attempted), (4) by establishing positive correlations between parents and offspring (Müntzing and Akdik, 1948), (5) by the study of genotypes unbalanced by supernumerary chromosomes, as in *Sorghum* (Darlington and Thomas, 1941).

Segregation for chromosome characters in the derivatives of a hybrid or inbred organism reflects genotypic control ; and the *manner* in which these characters segregate may be expected to reveal something of the nature of the control, *e.g.* whether monogenic or polygenic. Response to selection or the establishment of positive parent-offspring correlations for a particular chromosome character would also conclusively demonstrate genotypic control. Lastly, where extra chromosomes are used, both their influence and constitution can be determined, *e.g.* in *Sorghum* the supernumeraries which cause extra mitoses in the pollen are heterochromatic.

The following account concerns inbred rye, which is suitable

material for the purpose in that (1) the chromosomes are few ($2n = 14$), large and easy to handle, (2) the plants are economical to grow in terms of space and labour, and (3) under natural conditions the plants are virtually complete outbreeders.

A normal rye population is heterogenic by virtue of its outbreeding system : and its efficiency as a population will have been achieved by a selection for efficient heterozygotes. Experimental inbreeding gives rise to homozygous plants, a genetic condition which does not occur and hence is never exposed to selection under normal circumstances. In this enforced, unnatural, relatively homozygous state there is, theoretically, good reason to expect phenotypes that do not compare favourably with the population average ; the heterozygous genetic balance that had been built up by selection has been replaced by a homozygous condition that, being untested, is genetically unbalanced (Darlington and Mather, 1949). In practice this has been confirmed by following the progress of inbreeding in morphological, and also cytological, characters. Lamm (1936) was the first to show that the chiasma frequency in p.m.c. of inbred plants is characteristically lower than in population plants. In addition a relatively high proportion of p.m.c. in the inbred plants are structurally abnormal at meiosis. The fertility of inbred plants is reduced in consequence.

Inbreeding experiments should, however, do more than reveal the deleterious effects on the chromosomes that are due to the breakdown of the genetic system. They should show segregation for characters that are genetically controlled. This segregation would lead to increasing diversity between inbred lines, and at the same time to a greater uniformity of the plants within lines. Where this can be demonstrated for a nuclear property, genetic control is proved. This is the principle underlying the experiments to be described.

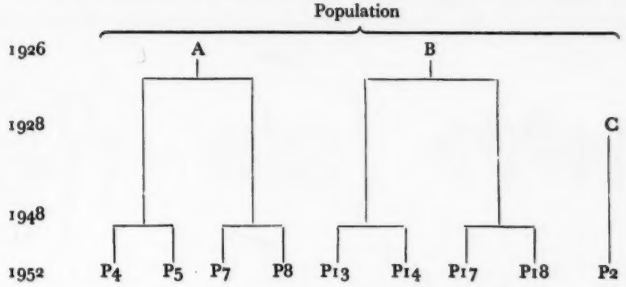
Line differences in chromosome pairing and contraction are indicated by Lamm's results in 1936. In 1948 Müntzing and Akdik showed that in the second and third selfing generations there are significant differences between inbred lines for one of the chromosome characters which they studied, namely the average number of chromosome arms paired per cell at first metaphase of meiosis. They found no line differences for other characters at meiosis. It is evident, however, from the work of Prakken and Müntzing (1942) on neocentric, or T-end, chromosomes, that line differences exist for other chromosome characters in rye. Inbred lines differ in the presence and in the intensity of neo-centric activity. While our chief aim is to inquire further into the effect of the genotype on chromosome behaviour in rye, the characters used for this purpose are in themselves instructive. They throw light on the causal sequences producing chromosome abnormalities, and they will therefore be described in some detail.

2. MATERIAL

The basic inbred lines described were taken at random from material kindly presented to the department by Professor Müntzing. They are derived from a population of the Swedish variety Stålrag, and their history is set out in table 1. Two pairs of inbred lines originated from two population plants (A and B) grown

TABLE 1

The history of the inbred lines (see text). A, B and C are the three population plants of the variety Stålrag, from which the lines were derived by self pollination



in 1926. The other line comes from a population plant (C) grown in 1928. All have been inbred by continuous self pollination. At Birmingham in 1948, the two pairs of inbred lines from A and B were split and sub-lines started from sister plants within each line.

3. METHOD

All the observations were made at meiosis in p.m.c., chiefly at first metaphase and anaphase. The chromosome characters followed will be described in the next section. Meiosis has been studied in five plants from each of the eight sub-lines derived from A and B, and in five plants in the line started from C. The fixations were made at intervals of about five days, from 17th June to 20th August 1952.

For cytological examination whole heads were fixed and mordanted in acetic alcohol containing a few drops of aqueous ferric chloride solution. After a week the heads were put into 70 per cent. alcohol and stored at 0° C. Squashing, and staining with aceto-carmine, then produced satisfactory preparations.

4. THE CHARACTERS

(i) *Numerical*

Chiasma frequency.—The scoring was made at metaphase in 20 p.m.c. in each plant. In squash preparations there is little difficulty in interpreting the bivalents, although some selection of the best cells has often to be made. The values are expressed as the average chiasma frequencies per cell.

Terminalisation.—It is usually possible to distinguish between the terminal and intercalary chiasmata with reasonable accuracy at metaphase, but the scoring is perhaps not as precise as for the frequency of chiasmata. The terminalisation values were obtained by expressing the terminal chiasmata per cell as a proportion of the total number of bivalent arms paired per cell. Such proportions give a truer

picture of terminalisation than when terminalisation is expressed in relation to total chiasmata. This is made clear in considering ring bivalents at metaphase with two chiasmata and three chiasmata. In each case the maximum number of chiasmata which could be terminalised is two. Yet, given maximum terminalisation in both types of bivalents, if this is represented in relation to total chiasmata the values for the two bivalents is different, 1 and $\frac{2}{3}$ respectively. When, on the other hand, the proportion is terminalised chiasmata to the total of arms paired, the two kinds of bivalents have the same value, 1 ; and this more correctly indicates the terminalisation

FIG. 1*a*, *b*, *c*, *e*.—First metaphase showing different degrees of chromosome pairing and coiling. *c*. Six univalents ; nucleic acid starvation near to the chromosome ends in the two rod bivalents. *d*. First anaphase where the chromosomes are poorly coiled. Two univalents have divided late. ×1100.

capacities of the two bivalents. The method is useful here where there is a large variation in the chiasma frequencies of the plants compared. The proportions derived in this way were then transformed to angular values so that the results could be treated by simple averaging (Mather, 1943).

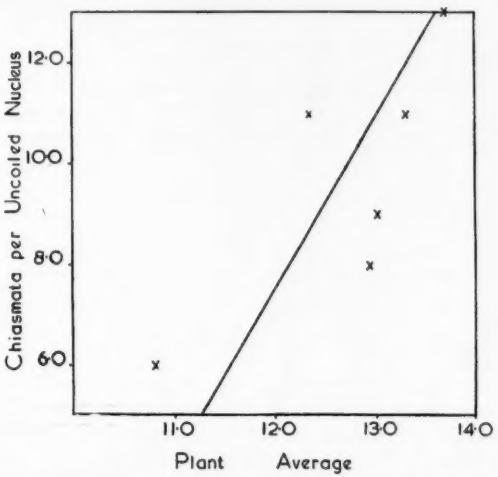
(ii) *Structural*

Uncoiled chromosomes.—The degree of uncoiling varies (figs. 1*b*, *c*, *e*). For the purpose of classification only the more extreme cases of uncoiling (as in figs. 1*c* and *e*) were taken into account. Cells with uncoiled chromosomes form about one per cent. of the total in one

anther, side by side with normal cells. Their chiasma frequencies are characteristically lower than the average for the anther (graph 1). Poor coiling and poor pairing suggest a reduced precocity of meiosis in these cells.

Uncoiling, however, is not always associated with low pairing. The different kinds of asynaptic cells indicate, as might be expected, more than one cause of pairing failure.

In a few cells where uncoiling is extreme a number of chromosomes show nucleic acid starvation near to the ends (fig. 1*c*). These starved areas correspond to the heterochromatin mapped by Levan (1942) in haploid and also in cold-treated rye.



GRAPH 1.—Relationship between the chiasma frequencies of single uncoiled nuclei and the plant averages. The regression is significant at approximately the 5 per cent. level.

Bridges without fragments.—Anaphase bridges of this kind are the result of splitting errors. The regions at which the chromosomes stick at anaphase are invariably confined to the ends or near ends of the chromosomes (figs. 2*a*, *b*, *c*, *f*). This localisation of error may well be controlled by the centromere in the manner deduced from the behaviour of X-rayed chromosomes in *Scilla* (Rees, 1953). Alternatively, the separation errors may coincide with, and be determined by, the heterochromatin near the ends (*cf.* cold-treated *Trillium* root tips, Darlington and La Cour, 1940). In some plants as many as 16 per cent. of first anaphases are affected. Similar bridges are common in inbred maize (Blanco, 1948).

In all cases the anaphase sticking involves two of the four strands (figs. 2*a*, *b*). The cause may be a local failure of reproduction of the chromosome thread or its nucleic acid sheath, or an abnormal chiasma

formation (Darlington, 1949). Failure to split can cause breakage by tension (fig. 2*c*).

In one plant one case of intercalary sticking was seen at second anaphase. It is probable that terminal splitting errors may be responsible for other bridges found at the second division in this and other plants.

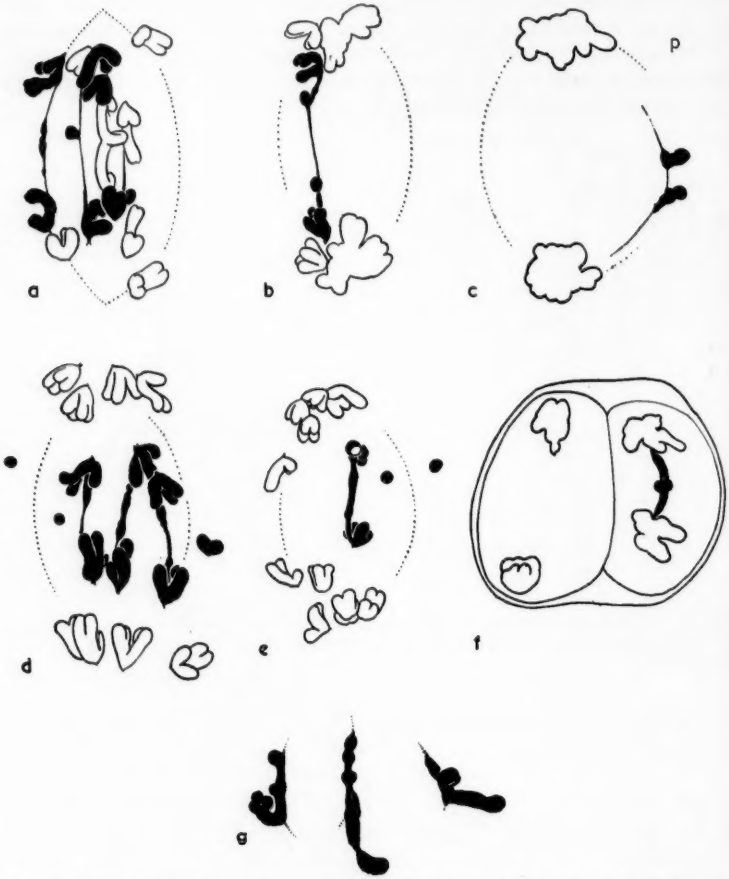


FIG. 2*a*, *b*, *c*.—Splitting errors at first anaphase. In *c* the tension due to the splitting error has resulted in breakage during separation. *f*. Intercalary splitting error at second telophase. *d*, *e*. Bridges and fragments at anaphase in P13. *g*. B″ breaks in P13 bivalents at first metaphase. The acentrics remain attached by chiasmata. One break is interstitial, the others at or near the centromere. × 1100.

Bridges with fragments at anaphase.—These are found in most plants (table 4). Rare bridges and fragments could possibly be explained by crossing over in segments heterozygous for inversions; for even

after prolonged inbreeding a small amount of residual heterozygosity might be expected.

In sub-line P13, however, each plant has 20-50 per cent. of its p.m.c. containing from one to four acentric fragments with or without bridges at first anaphase (figs. 2*d*, *e*). If this is due to inversions it means either :—

(i) high structural heterozygosity of the original population parent and intense selection against structural homozygotes, or
(ii) an accumulation of inversions by breakage and reunion during the course of inbreeding, and their maintenance in the heterozygous state by selection against the survival of homozygotes.

On either view it has to be assumed that the inbred plants are heterozygous for considerable lengths of the chromosomes. Evidence of two kinds will be provided that inversion heterozygosity is not in fact a satisfactory explanation for the bridges and fragments in this sub-line.

1. Sub-line P14 was derived from a sister plant to that from which sub-line P13 was continued, only four generations before this analysis (table 1). Yet in P14 7 per cent. is the maximum frequency of cells found to contain fragments and bridges at anaphase, the average for the five plants being less than 6 per cent. If P13 plants were inversion heterozygotes their persistence would have depended on the structural homozygotes being more or less lethal. There is no suggestion of lethality in P14 plants, yet the scarcity of bridges and fragments indicates that they are structurally relatively homozygous.

2. Four F_1s from crosses between plants of sub-line P13 and another line show fragments and bridges in a maximum of 2 per cent. p.m.c. Assuming P13 plants to be heterozygous for a number of inversions, the F_1s would be expected to show good evidence of these.

In the face of these facts inversion crossing over appears inadequate to explain anaphase bridges and fragments in P13.

" Intra-haploid pairing " could explain bridges and fragmentation (Darlington, 1937) ; but it is not likely in this case in view of the relatively high chiasma frequency in P13 which indicates good pairing. It may well be that in other lines where pairing is poor, bridges and fragments do arise from this cause.

Splitting errors account for some (less than 5 per cent.) of the bridges and fragments at first anaphase. The remainder, it is suggested, are due to a genetically controlled chromosome breakage (with or without reunion) at first prophase of meiosis, the acentric fragments being held in the bivalents until anaphase by virtue of coiling (*cf.* X-rayed meiosis in *Locusta*, White, 1937) and chiasmata. Reductional and equational separation for the points of sister reunion would give second and first division bridges respectively. Prophase breakage is

in fact detectable at metaphase in about 1 per cent. of P13 bivalents (fig. 2*g*).*

On this view, too, a divergence is indicated between sub-lines P14 and P13, but it need not be of the same order implicit in assuming that the bridges and fragments were due to structural heterozygosity. Indeed the divergence, with respect to this one character, could be due to the segregation of a single gene difference when the sub-lines were separated. It would of course imply heterozygosity of the parent from which the sub lines arose, or possibly, mutation subsequent to splitting the sub-lines.

Errors at the pre-meiotic mitosis.—Pre-meiotic disturbances have been inferred in inbred and population rye by Müntzing and Akdik (1948), Müntzing and Prakken (1941). The consequences of such irregularities have been studied in some detail at meiosis in one of our inbred lines.

Where pre-meiotic abnormality does occur it affects about one per cent. of the p.m.c. In these cells a number of extra chromosomes or fragments accompany the normal seven pairs. The extras range from one acentric fragment (fig. 3*c*) to many centric fragments and even complete extra bivalents and univalents (fig. 3*h*). All are enclosed in one cell wall. The presence of extra unbroken chromosomes and centric fragments indicates that a spindle abnormality accompanies breakage, so that the cell wall formed does not equally separate daughter nuclei. Cell walls occasionally fail to form independently of breakage. Then the p.m.c. are fully binucleate. But when this happens, one nucleus can often be distinguished from the other (fig. 3*h*) : at metaphase its chromosomes are less contracted and more faintly stained and have fewer chiasmata. Although the two nuclei share the same spindle, no multivalents occur, so that pairing must take place within separate nuclear membranes. This is true also where the extra chromosomes do not make up a full set, and micronuclei can in fact be seen separate from the normal nucleus at pachytene and early diplotene (figs. 3*a*, *b*).

Like the chromosomes of many extra complete nuclei, those of micronuclei can clearly be distinguished at metaphase from the normal set, and for the same reasons (figs. 3*c*, *d*, *e*). Thus even though the extra chromosomes, whether they are complete or broken, reach a metaphase condition simultaneously with those of the normal nucleus, their reduced coiling and staining, and comparative asynapsis indicate a delay at earlier stages. This in fact is what happens (figs. 3*a*, *b*). At diplotene and diakinesis the extra chromosomes and fragments are at a stage corresponding to pachytene. This initial delay must be followed by great acceleration in the division cycle of a micronucleus to enable its chromosomes to coil and be ready to move on to the spindle at the same time as those of the normal nucleus in the same cell. Initial delay of retarded nuclei followed by an acceleration of the division process has been inferred by Darlington

* Details of this breakage will be published in a later paper.

and La Cour (1945) in X-rayed *Tradescantia* pollen grains, and observed in p.m.c. of *Scilla* that were asynaptic and showed chromosome breakage (Rees, 1952). In *Scilla* it was suggested that the retarded nuclei were stimulated to division by the actively dividing healthy

FIG. 3.—Extra chromosomes and fragments in p.m.c. of P4 and P5. *a*, *b*. Retarded micronuclei at diplotene. In *b* the extra chromosome shows pairing at three places, including ends, between its own segments. *c*. Extra, thin acentric fragment at metaphase. *d*. Extras are univalents or fragments. *e*. Extra bivalent, univalents and fragments. *f*. Extra piece retarded at metaphase. *g*. One micronucleus retarded, the contents of another in a more advanced state of coiling on the metaphase plate. Note starved heterochromatin in the latter. *h*. Two complete nuclei in one p.m.c. One is distinguishable from the other, being less densely stained, relatively uncoiled and having only four chiasmata. ×1100.

nuclei surrounding them. The same can be inferred for the micronuclei in rye p.m.c. It is all the more likely in this case since the healthy and sick nuclei share the same cytoplasm, whereas in *Scilla* and *Tradescantia* they occupied different cells.

Delay followed by acceleration is true of most but not all micronuclei. In three out of about a hundred abnormal metaphases, the contents of the micronucleus have not caught up with the chromosomes of the normal nucleus. They still appear as early prophase chromosomes (fig. 3*f*). The question arises why some micronuclei can reach a metaphase condition and others not. The cytoplasms of different cells containing micronuclei might differ in constitution. Fig. 3*g*, however, shows both kinds of behaviour in the same cell. Evidently the explanation must be sought inside the micronuclei themselves.

The absence of a centromere in the contents of a micronucleus does not appear to prevent it from reaching a " metaphase " condition (*e.g.* fig. 3*g*). Neither does the absence of a nucleolus ; the majority of diplotene micronuclei lack one, yet the majority reach " metaphase ". There is evidence (John Innes Report, 1951) that *Vicia* and *Hyacinthus* micronuclei, produced by chemical and X-ray treatment respectively, keep going when they contain both a nucleolus and a piece of heterochromatin. In rye the nucleolus is ruled out, and it may be therefore, that heterochromatin is the essential constituent. Whether all the " successful " micronuclei contain heterochromatin is not known. That many do so can be seen where it shows up as starved bands (fig. 3*g*).

The metaphase configurations in cells with broken chromosomes are explained by chromatid (B′) breaks, with or without reunion, at the pre-meiotic mitosis. As the breakage occurs a division before the cells are examined, it is perhaps not surprising that the breakages seen are of the B′ type. If B″ (chromosome) breaks occur, both daughter nuclei will be deficient and less likely to undergo another cell division.

To account for the inclusion of unbroken chromosomes and centric chromosome fragments in micronuclei, it was suggested earlier that a spindle abnormality generally accompanies the breakage at the pre-meiotic mitosis. Since the micronuclei are found in cells in which the main nuclei are perfectly normal, one pole must have been a proper one, the other disturbed, and probably split. This suggests a gradient within the cell such that the cytoplasm at one end is actively different from that at the other end. Now, two complete nuclei in one cell, it will be remembered, are often phenotypically different at first metaphase. This is precisely what would be expected if a cytoplasmic gradient of the kind suggested exists. Such a gradient could account for the invariable delay of micronuclei at the early prophase of meiosis. The micronuclei are derived from the postulated disturbed pole of the cell. Their behaviour, which is similar to that of one of the two complete nuclei in binucleate cells, may reflect the persisting influence of their cytoplasmic environment in the parent cell. Intra-cell cytoplasmic gradients that differentiate nuclei are, of course, well known, particularly in pollen grains (La Cour, 1949 ; Darlington and Mather, 1949).

Mobile chromosome ends.—The property of active movement of certain chromosome ends in rye has been dealt with at great length by Prakken and Müntzing (1943), Ostergren and Prakken (1946)

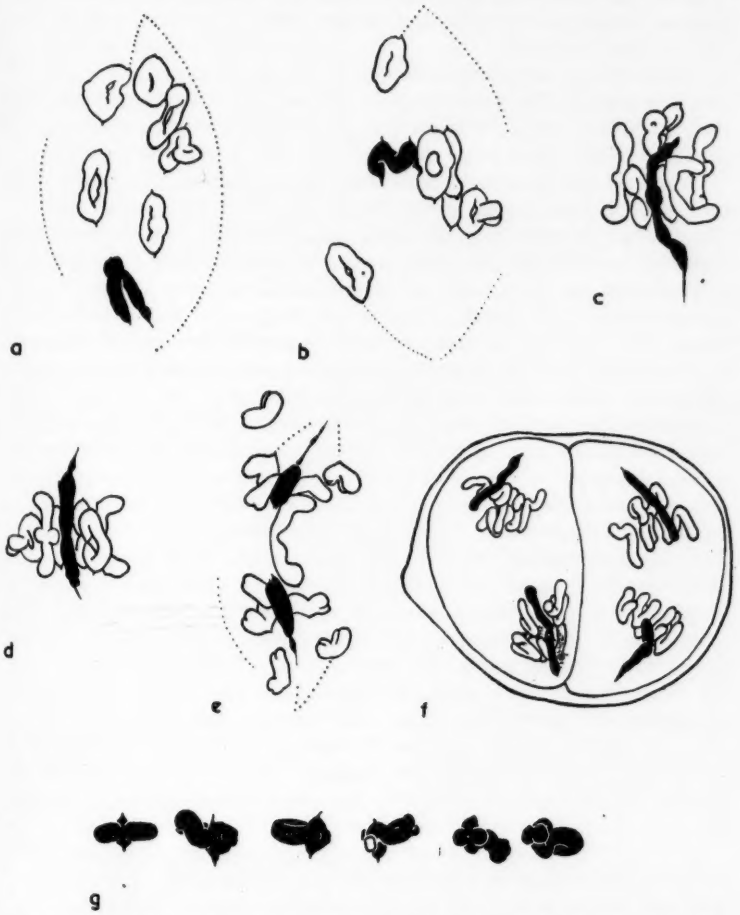


FIG. 4.—Neo-centric activity in P4 and P5. *a*. Prometaphase with the neo-centric ends of one bivalent directed to one pole. *b*. Co-orientation between neo-centric ends and centromeres of the same chromosome in a bivalent at prometaphase. *c, d*. Neo-centrics at first metaphase. *e*. First anaphase with neo-centric ends leading to the poles. *f*. Second anaphase with four neo-centric ends. *g*. P bivalents in P13. All are of the kind with sub-terminal centromeres. On the extreme left is one with a proximal and a distal chiasma in each arm. ×1100.

and Kattermann (1939). Similar cases are known in maize (Rhoades and Vilkomerson, 1942), and in *Bromus* species hybrids (Walters, 1951, 1952).

Active ends are to be seen at both meiotic divisions in sub lines P_4 and P_5 (figs. 4*a-f*). As Prakken and Müntzing point out, it is mainly the short arms of the chromosomes that show such activity (fig. 4*d*). Where they occur the neo-centric ends are most often found in one pair of chromosomes only, but in one plant they were seen in five pairs of the same cell.

Diffusion of substances secreted at the centromere to the ends of the chromsome has been given as the cause of neo-centric activity (see Rhoades, 1951). The physiological implications are discussed at length by Darlington (1951).

Proximal chiasmata : P. bivalents.—As a rule few chiasmata form near to the centromeres of rye bivalents. The differential distance (Mather's *d*, 1937) is long. In some plants, however, at first metaphase as many as one fourth of the p.m.c. have one bivalent (henceforth P bivalents), rarely two, in which chiasmata are found in roughly the first proximal quarter of the chromosome arms. This feature is confined to the three bivalents with centromeres relatively sub-terminal (fig. 4*g*).

Proximal localisation would result from an alteration in the points of initial contact between pairing homologues ; from distal, as in normal rye bivalents, to proximal in P bivalents (*cf.* Frankel, Darlington and La Cour, 1940). Distal as well as proximal contact is indicated in more than 90 per cent. of P bivalents where a second chiasma forms towards the end of the long arm. That P bivalents are always of the sub-terminal centromere kind demonstrates how the differential distance can vary independently within a chromosome set. Evidence will be provided for the genetic control of this character. Genetic control of the localisation of chiasmata is, of course, well known in *Allium* (Emsweller and Jones, 1945, and others), and in one species, *Allium nutans*, localisation has appeared, as in rye, by inbreeding (Darlington, 1937).

Out of 29 p.m.c. with one P bivalent, five showed the P bivalents lying off the metaphase plate. No cases of non-disjunction were found at anaphase, so that the P bivalents rarely fail ultimately to congress in time for proper separation. Their relative slowness in moving on the spindle indicates, however, that the close proximity of the chiasmata to the centromeres interferes with movement. This interference is comparable to the case in spiders (Revell, 1947). There the bivalents at meiosis move on to the metaphase plate in a sequence which is inversely related to the differential distance.

Giant cells.—Coenocytic cells occur in three lines. They represent less than one per cent. of p.m.c. and contain on average about six nuclei. The coenocytes result from cell wall failures, beginning two or three mitoses before the first meiotic division. Similar giant cells were found by Peto at meiosis in *Lolium-Festuca* hybrids (1933).

Evidence of spontaneous breakage : Acentric fragments at first metaphase.—These have been found (other than where they result from pre-meiotic breakage as described earlier) in rare single cells in very few anthers.

Bivalent interlocking was also noted.

5. METRICAL AND MERISTIC CHARACTERS

There is an important distinction between the characters listed as structural and those listed as numerical. This is made clear on considering pre-meiotic errors and chiasma frequency respectively as representatives of the two groups.

Where pre-meiotic errors occur the majority of p.m.c. within a plant anther are perfectly normal, the minority abnormal, *i.e.* showing extra chromosomes or fragments. The intra-plant variation is discontinuous, and two classes of cells exist side by side. This character, like others in the structural group, can be described as meristic.

In contrast, the intra-plant distribution of chiasmata per cell is a continuous one around the mean value. There is no sharp distinction between cell classes with few and with many chiasmata. The same holds for terminalisation values. These characters can be described as metrical. In the strictest sense, of course, chiasma frequency shows meristic variation in that there are many classes of cells in one anther, but as the differences between the cell classes are so small, the characters can conveniently be treated as metrical.

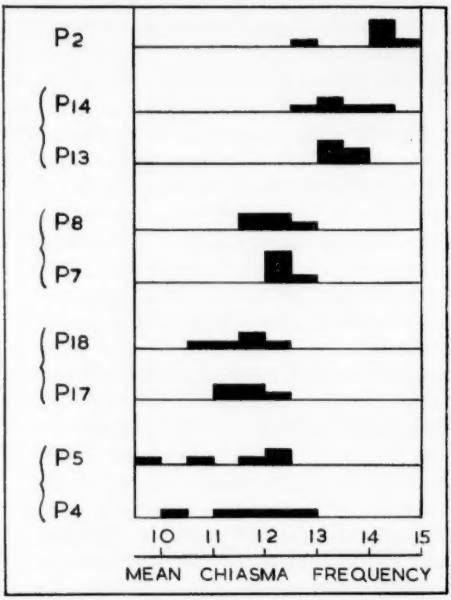
Since p.m.c. within an anther are genetically identical, the variation between them must be non-genetic, being due presumably to minute differences in conditions within the anther. As we have seen, there can be variation of more than one kind. There is continuous variation around the mean for metrical characters. For meristic characters the p.m.c. comprise two categories, normal and abnormal. The control of meristic characters is evidently explained by a threshold type of reaction that is common for structural abnormalities at meiosis (*cf.* Darlington and Upcott, 1941). The threshold determines whether the development of the p.m.c. will be phenotypically normal or abnormal. All the p.m.c. in the structurally abnormal plants are genetically potential variants from normal. Whether this potentiality is realised in any cell is decided by its immediate environment within the anther. Beyond a certain range of conditions it will be abnormal, within this range, normal.

There may evidently be an important difference between meristic and metrical characters in their response to selection. For metrical characters most p.m.c. will reflect more or less closely the genotypic potentialities of the individual. Where, for any reason, there may be a selection for or against the mean value of the character this selection will be effective in most of the cells. Response will be relatively quick. Contrast this with meristic characters, such as pre-meiotic errors. Here the control is such that the genetic potentiality for abnormality is realised only in a small proportion of p.m.c. Since selection acts on phenotypes, it is clear that whatever the advantage of the character in question, response to selection will be less effective because the phenotype of the majority of the cells in no way reflects their genetic difference from normal.

6. ANALYSIS OF THE INBRED LINES

(i) *Environmental variation*

Since the fixations for meiosis were extended from 17th June to 20th August, fluctuations in the weather may account for part of the variation observed in chromosome characters. There is no satisfactory way of eliminating this variation when, as in this instance, the plants are grown out of doors. The plants were sown at the same time and in so far as the different rates of maturity of the inflorescences, and



GRAPH 2.—The distribution of mean chiasma frequencies in the inbred lines. Each pair of sub-lines bracketed together was derived from the splitting of an inbred line four years previous to the analysis. Note similarity between members of pairs of sub-lines.

consequently the dates of fixation, affect the behaviour of chromosomes, the variation from this cause is in a sense heritable because the rate of development of the inflorescences is itself genetically controlled. This kind of variation is, however, as we shall see, of little importance in comparison with the variation due to genotypic control exercised more directly at the p.m.c. level. Nevertheless, an estimate of the amount of variation due to the effects of fixing on different days is desirable. This has been made, for chiasma frequency, by comparing plants from an F_3 generation that were fixed on seven separate days covering most of the fixation period, *viz.* 17th June

to 27th July.* An analysis of variance showed that the effect of fixing the plants on these separate days contributes very little to the total variation in chiasma frequency. It is not significant. On the strength of this evidence the method adopted will be based on the assumption that where differences can be shown between lines for nuclear characters they can be accepted with confidence as being predominantly genetic in determination.

TABLE 2

The average chiasma frequencies in 45 inbred plants. Each pair of sub-lines bracketed together is derived from an inbred line that was split four years previous to the analysis (see table 1)

Population ancestor	C	A				B			
Lines and sub-lines	P_2	P_4	P_5	P_7	P_8	P_{13}	P_{14}	P_{17}	P_{18}
5 plants in descending order of chiasma frequency	14·55	12·50	12·35	13·10	12·80	13·85	14·25	12·00	12·25
	14·30	12·15	12·15	12·45	12·10	13·80	13·70	11·75	11·65
	14·30	11·90	11·80	12·35	12·05	13·40	13·30	11·65	11·50
	14·10	11·00	10·80	12·35	11·70	13·30	13·05	11·45	11·40
	12·50	10·15	9·90	12·20	11·50	13·15	12·95	11·00	10·65

(ii) *Numerical characters*

Chiasma frequency.—The average frequency per cell is given for each plant in table 2. Five lines are represented, four of them by a pair of sub-lines each. The data are also represented in graph 2.

Below is an analysis of variance for differences between lines and between sub-lines within lines.

Item	SS	N	MS	VR	P
Between lines . . .	39·874	4	9·969	23·238	0·001
Sub-lines within lines .	0·594	4	0·149	...	...
Error	15·439	36	0·429	...	...
Total . . .	55·907	44	...	...	...

The differences in chiasma frequencies between lines are highly significant ($P = <0{\cdot}001$) when compared with the differences between individuals within lines. There is no doubt that the lines differ genetically in the control of this character.

* F_3 plants for the purpose of this estimation have three important advantages: (1) they are partly inbred, (2) they comprise a number of diverse families. They should therefore react to environmental changes more like the inbred lines than population plants used as a control. (3) The maturing of plants within F_3 families is not uniform to the same extent as in most of the inbred lines. Consequently, F_3 fixations on any one day, in contrast to those of the old inbred lines, tend to be a random sample of inbred plants from more than one family. Hence environmental variation can be more efficiently separated from the heritable and estimated.

There is no evidence of significant differences between the sub-lines within lines. At the time of splitting into sub-lines the four lines had undergone twenty-three generations of selfing. If there were significant differences between the sub-lines derived from any one line it would suggest some residual heterozygosity at the time of splitting. There is no evidence of this for the genes controlling chiasma frequency.

TABLE 3

The average terminalisation per cell in the inbred lines, expressed in angular values. The order of plants is the same as for table 2

Population ancestor	C	A				B			
Lines and sub-lines	P2	P4	P5	P7	P8	P13	P14	P17	P18
Average terminalisation	56	57	55	58	55	52	63	61	62
	53	54	56	57	57	54	60	64	61
	60	60	57	60	58	51	59	60	64
	59	54	56	58	56	51	61	65	64
	59	63	58	61	60	53	67	63	66

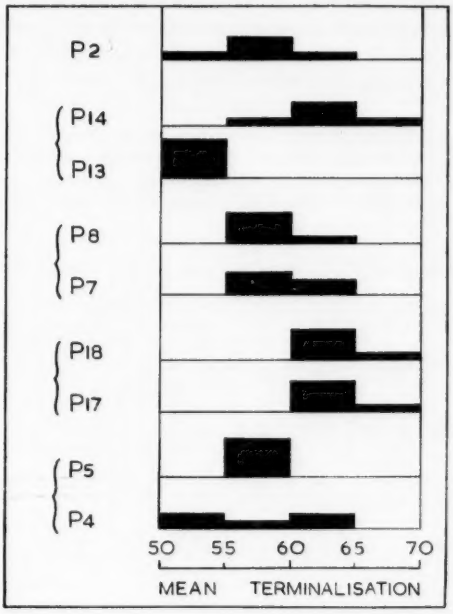
The analysis of variance follows :—

Item	SS	N	MS	VR	P
Between lines . . .	253·459	4	58·865	10·742	0·001
Sub-lines within lines .	249·285	4	64·821	11·829	0·001
Error	197·287	36	...	...	...
Total . . .	692·031	44	...	...	...

Terminalisation.—The values were first calculated as proportions of terminalised chiasmata to the total number of bivalent arms paired per cell. The proportions were then converted to angular values. For each plant, the average value per cell is calculated from 20 p.m.c. They are shown in table 3 and graph 3.

Differences between lines are again highly significant, but so also are differences between sub-lines within lines in this instance. The significant difference within lines is accounted for by sub-lines P13 and P14 (see graph 3). When the sum of squares for their differences (and the one degree of freedom relating to it) are taken away, the differences within lines are not more than would be expected by chance. The new mean square for within lines is then 4·737, a value smaller than the error mean square. The sum of squares for the difference in terminalisation between P13 and P14 is 245·075, the Variance ratio 44·722, and the probability for getting such a large difference by chance less than 0·001.

From this analysis of terminalisation values there is (1) clear evidence for genetic differences between lines, (2) evidence of a divergence between sub-lines P13 and P14 which suggests some residual heterozygosity of the line from which they were derived four generations earlier. Earlier, a genetic divergence between P13 and P14 was indicated with respect to anaphase bridges and fragments: this supports the view that the line at the time of splitting was heterozygous. Such heterozygosity could result from gene mutation, or



GRAPH 3.—The distribution of mean terminalisation values in the inbred lines. Note the difference between P13 and P14. No differences are indicated between members of other sub-lines.

from intense selection against homozygotes, or both. Residual structural heterozygosity in P13 was earlier considered to be extremely unlikely.

(iii) *Structural characters*

The distribution of abnormal chromosome characters in the inbred lines is shown in table 4. The method of demonstrating genotypic control will be essentially the same as for the numerical characters, namely to compare the variation between individuals within lines with the variation between lines.

Within lines.—It is clear from the table that plants within lines, and within sub-lines, are phenotypically heterogeneous for many of

these characters. Not only do plants within sub-lines and lines differ in that some do and others do not show a particular character ; there may also be marked differences in the expression of this character. For instance, in sub-line P_5 four plants out of five have p.m.c. with neo-centric ends at meiosis, the other has not. Of the plants with neo-centrics one has two, sometimes three, neo-centric end bivalents in some of its cells, whereas the others have not more than one in any p.m.c. Further, there is variation in the intensity of neo-centric activity. In some plants, the neo-centric activity is confined to a small proportion of p.m.c. and is so weak as to be hardly distinguishable ; in others it is strongly expressed in a high proportion of the cells.

TABLE 4

The distribution of structural abnormalities at first metaphase and anaphase in the inbred lines. Five plants from each sub-line were scored for the metaphase characters. Where fewer than five plants were scored for anaphase characters this number is given in brackets. The figures in the columns represent the number of plants in the appropriate sub-line in which a particular abnormality appeared

Lines and sub-lines	P_2	P_4	P_5	P_{13}	P_{14}	P_{17}	P_{18}	P_7	P_8
M1—									
Neo-centrics	...	5	5	...	...	...	...	...	...
Pre-meiotic breakage	...	4	4	...	...	...	...	...	...
P bivalents	4	...	...	5	1	...	1	...	...
Uncoiling	3	3	3	4	3	1	...	1	...
Acentrics	...	...	...	4	1	1	...	...	...
Giant cells	1	1	...	3	...	...	...	...	...
Interlocking	2	...	4	1	...	...	...	2	...
A1—									
Bridges	3 (4)	4 (4)	2 (4)	5	3 (4)	4 (4)	4	3 (4)	4 (4)
Bridges and ff.	4 (4)	4 (4)	2 (4)	5	2 (4)	3 (4)	5	3 (4)	4 (4)

Phenotypic heterogeneity of this order within an inbred line, however, does not necessarily mean that genotypic differences occur within it. Indeed heterogeneity may be expected for the structural characters in view of the threshold type reactions, described earlier, that control them. The threshold differentiates genetically equivalent nuclei within the same anther, and also causes differences between anthers. For example, one plant, $P4^4$, showed distinct neo-centrics in two anthers, none in another anther. All three anthers were fixed on the same day. In another plant, there were pronounced but rare neo-centrics in one anther, none in two others. These three anthers also were fixed at the same time. Evidently where this kind of variation exists between genetically identical cells, and anthers, of the same plant, it is not surprising to find phenotypic differences between plants of the same sub-line. In other words, the qualitative phenotypic differences are not in every case reflections of genotypic differences.

Between lines and sub-lines.—Bearing this limitation of classification in mind, genotypic differences between lines can be concluded where a character is restricted to, or significantly more frequent in, particular lines.

It is clear from table 4 that neo-centric activity and pre-meiotic errors in our sample are restricted sharply to P4 and P5. There is, too, undoubted heterogeneity with respect to P bivalents; they are found mainly in P13 and P2. Genotypic line differences for these three characters can be concluded with confidence. In sharp contrast some other characters, like splitting errors, are widespread throughout all lines and sub-lines. Considering the characters one by one, the probability of the lines being significantly heterogeneous for any one of them can be found by using a $2 \times n$ contingency table (see Mather, 1943). The analysis, in view of the smallness of the numbers involved, is not entirely satisfactory, but is nevertheless the most reliable in the circumstances. The analysis is given in full for neo-centrics (table 5).

TABLE 5

The contingency table for the distribution of neo-centric activity in the inbred lines

Lines and sub-lines	P2	P4	P5	P7	P8	P13	P14	P17	P18	Total
Neo-centrics .	...	5	3	...	...	...	...	...	...	8
No neo-centrics	5	...	2	5	5	5	5	5	5	37
Total .	5	5	5	5	5	5	5	5	5	45

The " between line heterogeneity " $\chi^2_{[4]}$ (there being five lines) is 34·053 and is highly significant ($P = <0{\cdot}001$).

The heterogeneity $\chi^2_{[4]}$ for differences between sub-lines within lines can be calculated by subtracting the $\chi^2_{[4]}$ for lines (34·053) from the $\chi^2_{[8]}$ for the nine sub-lines (36·791). This gives 2·738 for $N = 4$ and is not significant ($P = 0{\cdot}7\text{-}0{\cdot}5$). It is concluded, therefore, that, (1) lines are significantly heterogeneous with respect to neo-centrics, and (2) sub-lines within lines are not heterogeneous.

Shown overleaf (table 6) are the heterogeneity χ^2s and probability values for lines, and sub-lines within lines, for the " metaphase " structural characters. There is no suggestion of qualitative difference between lines for the anaphase characters and a test of significance is unnecessary.

The table shows that there are undoubted differences between the lines for the characters neo-centric ends, pre-meiotic errors and P bivalents (the probability in each case being less than 0·001). Line differences for acentric fragments and uncoiling are also significant, at the 0·01 level. The probabilities (0·2 to 0·1) are not significant for giant cells and interlocking.

For differences between sub-lines within lines there is significance at the 0·02 level with respect to interlocking. This is almost entirely attributable to the difference between P4 and P5. Their difference contributes a $\chi^2_{[1]}$ of 10·000 to the heterogeneity χ^2 of 13·125 for the sub-lines within lines. When this $\chi^2_{[1]}$ of 10·000 is removed, the $\chi^2_{[3]} = 3·125$ for the remaining sub-lines within lines and is not significant (P = 0·5-0·3). It is possible that P4 and P5 are genotypically divergent, but the possibility, in the absence of other evidence, must be treated with some caution. For P bivalents, although the $\chi^2_{[4]}$ for sub-line heterogeneity is not significant (9·203, P = 0·10-0·05), the individual heterogeneity $\chi^2_{[1]}$ for P13 and P14 is significant (9·148, P = 0·01-0·001). In view of the evidence already put forward indicating a divergence between these sub-lines, their heterogeneity with regard to P bivalents may well be genotypic. The heterogeneity χ^2s for the other characters are compatible with genotypic uniformity within lines.

TABLE 6

The χ^2 and probability values for heterogeneity between lines, and sub-lines within lines, for metaphase structural characters in the inbred lines. ($N = 4$ *in all cases*)

Character	Between lines		Between sub-lines within lines	
	χ^2	P	χ^2	P
Neo-centrics	34·053	<0·001	2·738	0·7-0·5
Pre-meiotic errors	34·054	<0·001	0	...
P bivalents	22·802	<0·001	9·203	0·1-0·05
Acentric fragments	15·577	0·01-0·001	8·654	0·1-0·05
Uncoiling	13·750	0·01-0·001	1·250	0·9-0·8
Giant cells	6·526	0·2-0·1	10·124	0·05-0·02
Interlocking	6·875	0·2-0·1	13·125	0·02-0·01

The lines can also be differentiated on a quantitative basis with respect to these structural characters. Thus 3 per cent. to 27 per cent. of p.m.c. in P13 and P14 plants have P bivalents. Elsewhere they occur in less than 1 per cent. of p.m.c. In the case of anaphase bridges and fragments, although they appear in all lines and sub-lines, their frequencies differ. In P13 plants 100 first anaphases contain, on average, 37 fragments (never less than 20). In the 40 other inbred plants the average for 100 cells is much less than 10. In P13 it has been inferred to be the result of a chromosome breakage not due to inversion crossing over, whereas inversion crossing over, and intra-haploid pairing, probably account for the comparatively rare anaphase fragments in other inbred lines.

The following conclusions can be drawn for the structural characters in the inbred lines :—

1. There are highly significant differences between the lines for (*a*) neo-centric ends, (*b*) pre-meiotic errors, (*c*) P bivalents.

2. There are probable line differences with respect to, (*a*) uncoiling, (*b*) acentric metaphase fragments.

3. Line differences are also indicated by variation in the frequencies of occurrence of (*a*) P bivalents, (*b*) anaphase fragments. The cause of anaphase fragments also is probably different in at least one of the sub-lines, P13.

4. There is no evidence of heterogeneity for (*a*) giant cells, (*b*) bivalent interlocking, (*c*) splitting errors (anaphase bridges without fragments).

The pattern of abnormalities.—Table 4 shows not only that particular abnormal characters are more, or less, frequent in some lines than in others. It shows also how each line tends to have its own particular pattern of abnormalities. Thus metaphase of meiosis in P7 and P8 is relatively regular, other than for occasional uncoiling and interlocking. In P4 and P5 meiosis is irregular in various ways ; so, also, in P13 and P14 but in clearly different ways. The different patterns emphasise, first, the divergence between lines, and, second, that the irregularities, following the breakdown of the efficient control of meiosis by inbreeding, occur in many combinations, any one of which is characteristic of one inbred line.

7. THE BREEDING SYSTEM, THE GENOTYPE AND THE CHROMOSOMES

The results show that chromosome behaviour at meiosis in rye is undoubtedly subject to control by the genotype. This has been demonstrated for chiasma frequency, localisation and terminalisation, and for a number of structural abnormalities.

From the continuous variation shown for chiasma frequency (*cf.* Müntzing and Akdik, 1948) and terminalisation (see tables 2 and 3, and graphs 2 and 3) it would appear that the control of both characters is polygenic. This does not mean that major gene differences governing pairing do not occasionally arise in a rye population. An " asynaptic " gene has in fact been inferred by Prakken (1943). For some structural characters, pre-meiotic errors and neo-centric activity, the pronounced qualitative differences between lines could mean that few genes are involved. Alternatively, these characters may be manifested only by genotypes with a limited number of the possible polygenic combinations that emerge with inbreeding.

Structural abnormalities, and also poor pairing, are rare in population plants and in F_1s (Thompson, unpub.) as compared with inbred plants. Hence, whatever the precise genetic mechanism, their control is closely influenced by, and related to, the mating system, and, consequently, to the amount of heterozygosity. An outbreeding rye population by virtue of the high average heterozygosity of its members is genetically balanced, and buffered against these disadvantageous irregularities. This genetically heterozygous buffer has

no doubt been built up, encouraged by selection, under natural conditions of outbreeding. Its breakdown coincides with the breaking down of the normal outbreeding system. With increasing homozygosis the abnormalities emerge, their particular form and distribution, as we have seen, being governed by segregation so that, although in the inbred plants in general the chromosomes work less efficiently, in families and in lines they may be less efficient in different ways.

While differences between p.m.c. nuclei in different lines are genotypic, variation between p.m.c. nuclei within a plant is a result of divergence in development of genotypically similar nuclei. Local environmental variations, acting we may suppose, through the medium of the cytoplasm, stimulate the divergence ; frequently, by virtue of threshold type reactions, into two sharply distinct classes of nuclei. Thus, intracellular cytoplasmic gradients, it was earlier suggested, are associated with pre-meiotic errors, initiating differentiation into normal and abnormal daughter nuclei at the pre-meiotic mitosis. Cytoplasmic gradients could equally well account for differentiation between p.m.c. normal and abnormal for other structural characters. The origin of such a gradient would be partly a property of the genotype, as well as the result of the environmental variation within the anther.

We have seen that p.m.c. nuclei at the same stage of development may be differentiated spatially within the anther. Differentiation in time also affects the action of genes causing abnormality. Whereas the nuclear abnormalities in inbred plants arise mainly at meiosis, P_4 and P_5 nuclei regularly go wrong at an earlier stage of development, the pre-meiotic mitosis. Gene action determining the nuclear phenotype then is dependent on the cytoplasmic environment of the nucleus, which changes in response to external stimuli, and in the normal course of differentiation during development.

A vital aspect of the genetics of chromosome behaviour is the relationship between chiasmata and genetic variability in a population. At the nuclear level the adjustment of chiasma frequency and localisation controls recombination, thereby regulating the release of genetic variability. Frequency and localisation of chiasmata, we know, are genetically determined. More knowledge of the details of their inheritance and, in particular, of their behaviour under selection, is a necessary future step to a better understanding of the genetic system in a population.

Finally, the investigation of the variation in chromosome phenotypes between individuals, between tissues, between cells within individuals, and even within nuclei offers a unique opportunity of defining more clearly the relations between genotype and phenotype, not only in heredity, but also in development.

8. SUMMARY

1. Meiosis in inbred rye is less efficient than in population rye. Inbred lines differ with respect to the following nuclear characters in the anthers :—

(i) Frequency, terminalisation and localisation of chiasmata.
(ii) Neo-centric activity.
(iii) Pre-meiotic errors.
(iv) Chromosome breakage at first prophase.

2. Variation between inbred lines indicates genotypic control of the nuclear phenotype. Chiasma frequency and terminalisation are polygenically controlled.

3. A divergence is shown between two sub-lines taken off a line that had been inbred by selfing for more than 20 generations. This is explained either by residual heterozygosity of the parent line, or by mutation.

4. Pre-meiotic errors, found in one line, are confined to about 1 per cent. of p.m.c. within an anther. The abnormal cells each contain a micronucleus, or occasionally a complete nucleus, in addition to the normal nucleus. The chromosomes of micronuclei are frequently broken ; they are invariably retarded during first prophase ; and they have a reduced chiasma frequency. At the end of prophase their division cycle is accelerated.

5. The origin and behaviour of the micronuclei are inferred to result from intracellular gradients in the cytoplasm of abnormal pre-meiotic cells. The gradients initiate differentiation between normal and abnormal cells, as well as between normal and abnormal daughter nuclei within the cells. Since, initially, antecedents of p.m.c. within an anther are genetically identical, variation between p.m.c. reflects environmental fluctuations affecting development.

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THE TIMING OF MITOSIS AND MEIOSIS IN *ALLIUM ASCALONICUM*: A PROBLEM OF DIFFERENTIATION

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1. THE ABNORMAL PLANT

TOGETHER with a fertile crop of onions raised at Benares, one plant was seen to set no seed in the glasshouse. The bulb was split and all the sets flowered in England. Their anthers, however, never burst. When ripe they turned green instead of yellow. Sections cut by the freezing microtome showed much chlorophyll in the anther wall. Within the anther the pollen grains dried up and died after their first mitosis. The development of the embryo sac was normal. With foreign pollen, moreover, the plants set seed which germinated well.

Thus the sterility of our plant, or clone as it now is, is due to failure of the pollen to develop to maturity. This decisive and total disability is preceded, however, by certain small and sporadic errors much earlier in the development of the pollen-forming tissue. From a study of these errors there came to light an instructive group or syndrome of defects whose origin we have attempted to trace.

2. METHODS

Anthers and root tips were fixed in fresh acetic-alcohol (1 : 3) for ten minutes and stored overnight in 95 per cent. alcohol before staining by the Feulgen method (La Cour, 1947). Equally good results were also obtained by fixing anthers overnight in acetic-alcohol with a trace of ferric chloride followed by staining in weak acetocarmine (P. T. Thomas, unpub.).

For studying their development the ovules and anthers were fixed in La Cour's 2 BE after a short treatment with Carnoy's fluid. The material was embedded and sections cut at 10-14 μ were stained by the Feulgen method. Light green was used as a counterstain for the cytoplasm and the cell walls.

3. MEIOSIS IN THE ANTHERS

The chromosome complement as seen in the root tips consists as normally in *Allium ascalonicum* of eight pairs of chromosomes of which two pairs—one longer than the other—had median and the rest sub-median centromeres. The latter class included a satellited pair carrying the nucleolar organiser (plate, fig. 1).

In the anthers of most of the flowers from different inflorescences meiosis is regular. In the remainder, about one-third, up to 20 per cent. of the pollen mother cells in every anther show a group of meiotic abnormalities including asynapsis.

(i) *Chromosome breakage*

Pre-meiotic cells.—In order to trace the causes of asynapsis to an earlier origin we examined the mitoses before meiosis. Sections from anthers at the pre-meiotic mitosis reveal zones of abnormal cells. The zones are always central and at the two ends of the lobes. Sometimes one zone is abnormal, rarely both. The abnormality consists in a delay in mitotic phase correlated with a larger size of cells and breakage of the chromosomes (plate, fig. 2). Evidently these cells have continued growing while the normal cells have stopped or slowed

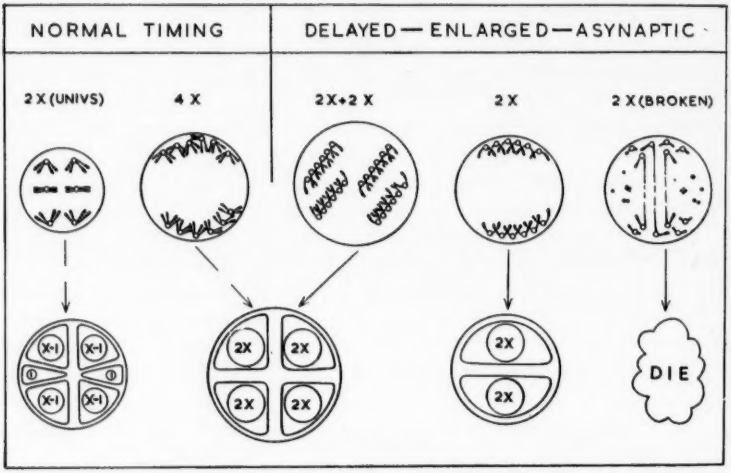

FIG. 1.—Diagram showing the chief courses of development in the two series with normal and delayed timing. The haploid set of eight is represented by three chromosomes.

Above : first anaphase, which in the asynaptic series is the only anaphase.

Below : the products of meiosis.

Note that tetraploid cells and nuclei occur in both series. Tetraploid nuclei with pairing and tetraploid cells (with two diploid nuclei) without pairing, both give four diploid pollen grains.

down their growth and entered mitosis. And the delay in mitosis (or the continued growth) is associated with a breakdown in chromosome development leading to breakage during the preceding resting stage.

All this happens two resting stages before meiosis. During the resting stage immediately before meiosis there are cells with micronuclei evidently derived from this fragmentation. They seem to disintegrate rapidly, however, for they are never seen among cells entering meiosis. Multinucleate cells (to which we shall return later) are found at this stage but they are either cells such as would be derived from failure of wall formation or from split spindles. They arise from spindle errors and not from chromosome errors.

Pollen mother cells.—The damaged pre-meiotic cells having disappeared, a second wave of abnormality strikes the meiotic cells. Presumably, although our squashes do not show it, the sensitive zones are the same zones as those affected earlier but in previously unaffected lobes. Again the affected cells are delayed in their development. The delay varies and during prophase is reduced. But again it is always associated with a larger size of the cells (plate, fig. 8). Again it seems that the delayed cells go on growing while those entering prophase cease growing or grow more slowly (fig. 1).

The delayed cells mostly show breakage, usually heavy breakage, at the diplotene stage (plate, figs. 4, 5). At first metaphase the cells with breakage reveal their abnormal conditions sometimes by stickiness of the chromosomes and sometimes by incomplete spiralisation, both of them typical of X-ray effects (plate, fig. 10). These combined with the breakage usually make analysis impossible (plate, fig. 7). Minute fragments, dicentrics, rings and sister reunions seen at first metaphase indicate that breaks are all of B″ type and have taken place before the end of pachytene. These breaks should be followed by reunions of chromosomes, or failing these, by sister reunions of chromatids. A preponderance of the results of sister reunion is noticed at anaphase (plate, fig. 9). This indicates that ordinary reunion, which precedes sister reunion, has been prevented, probably because the breakage takes place during pachytene when free movement is excluded.

Breakage in the delayed cells is not, however, invariable. Their invariable property is a greater or less degree of asynapsis. Thus the same abnormality leads to asynapsis and breakage but breakage must be supposed to demand a greater departure from normal.

(ii) *Asynapsis*

The asynapsis is of the maize type. At pachytene pairing is complete ; but by early diplotene a striking change has taken place. The points where the two chromosomes of each bivalent would normally cross over can then be seen to be simple coilings : chiasmata are absent (plate, fig. 4). This becomes even clearer at diakinesis as the coiling partly undoes itself (plate, fig. 5). By first metaphase, when the chromosomes have reached maximum contraction, the univalents have invariably become free and lie parallel.

In the anthers of our clone there is a sharp cleavage between the normally and the abnormally developing types of cell, just as earlier there is a sharp demarcation between normal and abnormal zones. The majority of the abnormal cells are completely asynaptic (plate, fig. 6). Only a few show numbers of bivalents varying up to four.

In the pollen mitosis is usually normal. Pollen grains are seen with varying numbers of micronuclei carried over from the breakage of meiosis (plate, fig. 18) but these never divide. Only seldom are

mitoses seen with fragments (plate, fig. 16) and still more rarely with reunions (plate, fig. 17). We see again, therefore, that the abnormal cells derived from breakage and asynapsis are eliminated between mitoses : natural selection takes effect.

(iii) *Polyploidy*

In our *Allium* clone the commonest method of giving rise to unreduced or diploid pollen grains is the substitution of a single division of unpaired chromosomes for the two meiotic divisions. Several other methods, however, are seen operating :

Spindle failure.—Occasionally at pre-meiotic mitosis the spindle mechanism totally fails. As a result the affected cells enter meiosis with a tetraploid complement. They are easily distinguished from the diploids at the pachytene stage (plate, fig. 3). At first metaphase, 16 bivalents can be seen and 16 chromosomes in each cell of the dyad at second metaphase (plate, fig. 11). At the end of meiosis tetrads of diploid pollen grains are formed.

Failure of wall formation.—In other cases the spindle at pre-meiotic mitosis fails only in regard to wall formation. This is evident from cells which enter meiosis with two diploid nuclei in each (plate, fig. 3). Such cells seem to give rise to diploid pollen grains in the following ways :

(i) When chiasmata are formed the 16 bivalents, 8 from each nucleus, are arranged on a common plate at the first metaphase. The rest of meiosis is, no doubt, completed in the normal way to give tetrads of diploid pollen grains.*

(ii) When no chiasmata are formed, that is, with asynapsis. The univalents, 16 from each nucleus, are arranged on two distinct metaphase plates. They will divide once to give four diploid pollen grains. In the binucleate pollen mother cells the stages of division in the two nuclei are always synchronised.

(iv) *Nucleolar microcytes*

In the normal series of cells a common feature is the occurrence of two univalents at first metaphase. While the bivalents divide and their halves pass to the opposite poles, these univalents lag near the equator without dividing. They may or may not be included in one or both of the anaphase chromosome groups. As a result the second division cells may have seven, eight or nine chromosomes and between them there may be one or two single chromosomes. These form one or two separate microcytes, cut off near the equator of the first division (plate, fig. 12).

* A parallel situation occurs in *Tradescantia paludosa*. Here binucleate pollen grains are formed owing to failure of wall formation at the end of second meiotic division. But the metaphase chromosomes fuse to give a single plate in the first pollen mitosis, which yields diploid generative and tube nuclei (Haque, 1953).

The microcytes are of interest because often their nuclei containing single chromosomes are seen to divide (plate, fig. 12). At the end of meiosis the microcytes therefore show one or two small nuclei in each depending on whether they have divided or not (plate, figs. 13, 14, 15).

The microcytes at the end of the first or during the course of the second division possess their own cytoplasm and a thin wall. Thus they are supernumerary separate cells with very defective nuclei in comparison with the two major cells of the dyad or four cells of the tetrad. Nevertheless the nuclei of the microcytes divide and they are always synchronised with the nuclei of the two major cells. Such a division has also been described in polyploid *Hyacinthus*, where the illustrations show the same synchronisation (Darlington, 1929). This is an example of co-operation between thin-walled cells in some sort of communication : the complete nucleus of one helps the incomplete nucleus of the other to undergo mitosis. In the absence of this co-operation the incomplete nucleus would not be expected to divide (Barber, 1941 ; Haque, 1953).

The two chromosomes whose occasional failure of pairing leads them into microcyte formation seem to be always the two nucleolar chromosomes of the complement. At second anaphase their satellites show very clearly. They can then either be seen in the microcyte or missed from one or both the cells of the dyad.

Nucleoli appear not only in the microcytes of a tetrad but also in all the four major cells. Thus, as McClintock (1934) found in maize, in the absence of a specific nucleolus-organising chromosome its function is undertaken by some other chromosome or by all the chromosomes of the complement.

On rare occasions tetrads are seen with more than two microcytes. In an extreme case as many as eight microcytes have been seen, but in these none of the micronuclei ever divide. Evidently chromosomes other than the nucleolar one may also exceptionally fail to pair. Some second meta-anaphase figures were in fact seen where 10 and 6, or 11 and 5, chromosomes constituted the two plates. But only those solitary chromosomes with the organiser form temporarily viable supernumerary cells.

Two questions now arise. (1) Why have the nucleolar chromosomes a special propensity for failing to pair or form chiasmata at prophase of meiosis ? (2) Why have they a special capacity for forming microcytes which can undergo an independent but synchronised second division ?

The failure of chiasma-formation, it would seem, must be related to the delay in pachytene pairing noted in the nucleolar chromosomes of *Fritillaria* (Darlington, 1935). The viable microcytes must be related to those seen in *Hyacinthus*. It is not known whether the chromosomes forming these separate cells and nuclei in *Hyacinthus* were nucleolar. But McLeish's discovery (1954) that fragments

containing the nucleolar organiser in *Vicia faba* can form viable micronuclei while non-nucleolar fragments at once die makes this probable. In the behaviour of nucleolar microcytes we thus see the consequences of mechanical and physiological properties of the nucleolar organiser which are quite unrelated in cause but happen to reveal themselves in a convenient order for observation.

4. THE PROBLEM OF DIFFERENTIATION

In the normal development of the anther in flowering plants there is a differentiation between tapetal and sporogenous tissue. But within the sporogenous tissue all differentiation is suppressed until after meiosis. It then occurs only between genetically identical cells *within* the several pollen grains. Differences at the same time arise *between* the pollen grains but these are differences of life and death. They should not (in our opinion) be ascribed to differentiation in a strict sense, for they are due to genetic differences between individuals. They arise from genetic segregation at meiosis as Barber showed (1941, 1942) by comparing the results of its suppression, artificially in *Uvularia*, and naturally in the Orchidaceae.

Now the symptom of homogeneity in the anther (and also in the testis) is the synchronisation of mitoses within the follicle before meiosis. The symptom of differentiation (or of segregation) is non-synchronisation, or more precisely, *dyschronisation*. Thus, when it happens that two dyschronised groups of cells arise before meiosis in the sporogenous tissue of the anther instead of one synchronised group, we have in a simple form the problem of differentiation. Our abnormal sterile clone has produced something more elaborate than arises in the normal development of the anther. This is not entirely novel but the significance of previous instances seems to have been overlooked.

Owing to the habit of studying pollen mother cells and pollen grains in smears we know very little about the positions in the anther of abnormally developing cells. The dwarf pollen grains lie at random in the *Tradescantia* anther (La Cour, 1949). But we do not know exactly where the embryo-sac-like pollen grains arise in the anthers of *Hyacinthus* (Stow, 1930).* We observe, however, that our sensitive zones lie in the middle of the anther lobes, in a symmetrical position corresponding to the embryo-sac in an ovule. The same seems to be true of the abnormal cells in *Scilla* : they are in the " mid-regions of the loculi " (Rees, 1952).

So much for analogy. So far as physiological principles are concerned it is clear that homogeneity in a tissue must always depend on free diffusion. Conversely the division of one kind of tissue into

* Stow found that diploid hyacinths (probably " La Victoire ") heated to 30° C. for 15 hours on 27th October and kept at 20° thereafter gave on 12th December together with 90 per cent. dead pollen grains a proportion of giant pollen grains with 8, and also 4 and 16 nuclei corresponding to the chief types of Angiosperm embryo-sac. These dry up and die as the anther ripens.

two kinds must always depend on the creation of a barrier to diffusion between them. In most flowering plants such a barrier arises later around every pollen grain. But it may come too late as in the Orchidaceous types of development described by Barber (1942). In *Scilla*, in *Chrysanthemum*, in our clone of *Allium*, and perhaps in other forms of male sterility, we may say that it comes too early.

The strength of the barriers to diffusion which will be effective should vary with the structure or function of the particles diffusing. In *Uvularia* and the orchids we indeed find that pollen grain walls are necessary to separate the cells physiologically and cause those which are genetically defective to lag in development. But in a case in *Tradescantia* (Haque, 1953), apparently as a result of a genetic difference, dyschrony sets in between nuclei in the same pollen grain. Here the effective or discriminate particle can have been stopped by no obstacle but the nuclear membrane itself.

Thus we have the nuclear barrier in *Tradescantia*, the absence of cell barriers after meiosis in orchids, and the premature creation of cell barriers before meiosis in the abnormal *Allium* or *Scilla*, all constituting exceptions which prove and define the ordinary and highly adaptive rule of development. This rule may now be stated as follows :

(i) No differentiation arises in the sporogenous tissue.

(ii) Effective barriers between cells arise after meiosis.

(iii) They make it possible for the genetically distinct products of meiosis to be separately tested in respect of their capacities for growth before they are committed to, and protected by, fertilisation.

5. THE BREAKAGE-ASYNAPSIS SYNDROME

The abnormal characteristics of our clone of *Allium cepa* are evidently genetically determined since they distinguish it from the normal in successive years and in different countries. The nucleolar microcytes and the polyploid cells were apparently independent in their origin of the main correlated or concurrent group of abnormalities. This group differs from the classical instance described by Beadle in maize in three respects :

(i) The sporogenous tissue is differentiated, as we have seen, into sensitive and non-sensitive zones in respect of breakdown before meiosis.

(ii) The sensitive zones reveal their defect in successive stages and doubtless early, or late, or not at all, subject presumably to conditions external to the tissue.

(iii) The defect reveals itself also as a group of errors delaying the onset of mitosis or meiosis, prolonging the growth of the cell, and upsetting the continuity or reproduction and pairing of the chromosomes.

Such a group of correlated abnormalities we may call a breakage-asynapsis syndrome, but it is, of course, a part of the larger syndrome which later brings atrophy to all the pollen grains after their mitosis.

In its general character our abnormality resembles that found by Rees in *Scilla*. It even shows the same preponderance of sister reunions after breakage at meiosis. The only differences are, first, that our asynaptic cells do not all show breakage so that we are able to separate the two errors. And, secondly, owing to the abundance of the *Allium* flowers, we are able to follow our abnormality back in development to an earlier stage and see its origin in two steps.

Both the *Scilla* and the *Allium* abnormalities agree with that found by Dowrick in *Chrysanthemum* in showing a correlation of enlarged cells and delayed prophase of meiosis with a reduced frequency of chiasma formation. In all three cases, but sharpest in *Scilla* and *Allium*, it is the differentiation of two types of mother cell in the same anther which makes it possible to demonstrate this multiple correlation.

The primary characteristic of the breakage-asynapsis syndrome is a delay in the onset of prophase of the pre-meiotic mitosis. This is followed by a corresponding delay in the later stages of this mitosis. But when it affects meiosis itself the delay is reduced by the time first metaphase is reached : the delayed cells partly catch up the normal ones. This principle of *convergence* in cell development was first inferred in X-ray experiments on pollen and was assumed to be due to diffusion of mitosis-promoting materials which would favour synchronisation (Darlington and La Cour, 1945, graph 11). The delay in the beginning of prophase is used, we must however suppose, in growth since the delayed cells are always larger than the normal ones. Evidently, therefore, the ordinary prophase represents a switch from processes of growth to processes of transformation, reconstruction and movement (fig. 2).

The suggestion that the beginning of prophase goes with a radical change, a switch, in cell metabolism rests here on an entirely new kind of evidence. But it is related to the evidence of Lettré (1950) and later of Stich (1954) that prophase is initiated by a change from aerobic to anaerobic respiration. Whatever the nature of the change, however, the organisation of mitosis or meiosis must represent a dissipation of energy and a loss of materials which would arrest or diminish any process of growth.

The secondary characteristic of the syndrome is its effect on pairing at meiosis. A postponement of prophase reverses the normal advancement of prophase which distinguishes meiosis from mitosis. It is natural therefore that it should counteract or undo some of the properties of meiosis. This it evidently does when it inhibits chiasma formation and metaphase pairing, producing " asynapsis ".

The habit by which the abnormalities affect only a part of the cells and these only in a succession of stages points, as we have argued, to a divergence of two types of cells following normal and abnormal

channels of development. It seems that the abnormality diverges to reach a threshold for breakage at different stages in the development of the pollen mother cell precursors. It also seems that the threshold for asynapsis is lower than for breakage since all delayed cells are asynaptic but not all asynaptic cells show breakage.

Several problems remain to be considered. The postponed prophase does not stop pachytene pairing which seems to be complete. It does not even prevent the chromosomes at pachytene developing

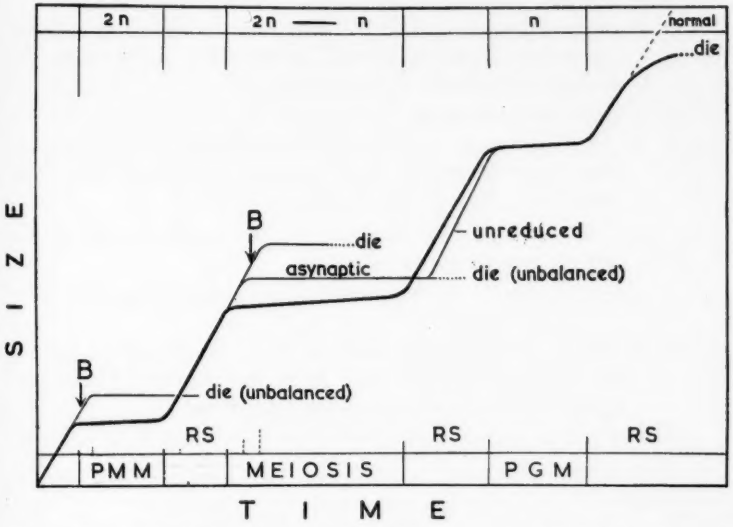

FIG. 2.—Graph showing the apparent relationships of growth of the male-sterile clone with stage of mitotic development in the general sporogenous tissue (thick line) in the anthers and in the sensitive zones as they become delayed and susceptible to breakage and asynapsis (thin lines). The death of all pollen grains is shown stopping growth in contrast to the development in the anthers of a normal plant.

a coiling which indeed survives at diplotene. It merely reduces the sequel of pairing and coiling, which is chiasma formation, to a very small fraction, less than one-tenth, of normal.

How are we to understand this? Clearly it is not that the chromosomes have become resistant to the breakage involved in crossing over since a proportion of them actually show spontaneous breakage. Rather it would appear that the coiling fails to reach the threshold torsion for crossing over. Observations of diplotene in normal meiosis generally suggest that only about half the coiling is used up in crossing over. A reduction of coiling to about one half such as might follow a delay in pairing would then give the typical asynaptic situation in which cells show a statistically normal curve of chiasma frequency but one in which the modal frequency has fallen to zero.

The study of the whole development of other types of abnormal anther in its spatial relations would no doubt reveal significant evidence on the physiological questions we have discussed.

6. SUMMARY

1. In the anthers of a male-sterile clone of *Allium ascalonicum* central zones of cells suffer a delay in the onset of mitosis or meiosis relative to the main body of cells.

2. The delayed prophase is correlated with three other effects :—

(i) Continued growth : thus the initiation of mitosis or meiosis represents a switch in cell metabolism, a diversion of energy from growth to reconstruction.

(ii) Chromosome breakage.

(iii) At meiosis, owing to failure of chiasma-formation, asynapsis even more regularly than breakage : thus a delay in prophase undoes the character of meiosis.

3. The undelayed cell development in the anther entails three successive and apparently unrelated errors :—

(i) Errors of spindle development lead to polyploid mother cells and pollen grains.

(ii) Nucleolar chromosomes show a special frequency of failure of chiasma-formation and form microcytes. These are capable of a synchronised second division.

(iii) All pollen grains shrivel and die after their first mitosis.

4. The whole syndrome of effects (*cf.* fig. 2) may be represented as follows :—

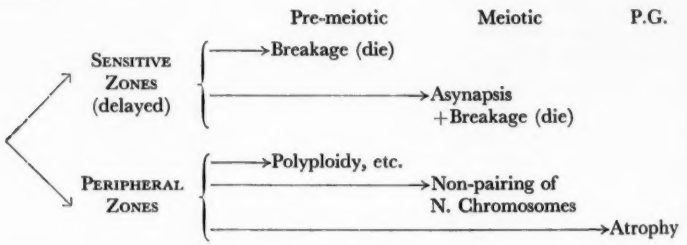

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Plate I

FIG. 1.—Metaphase from the root tip meristem ($2n = 16$). ×1170.

FIG. 2.—L.S. through anther. Most of the cells have completed the premeiotic mitosis and are at premeiotic resting stage. A group of delayed and broken cells are seen undergoing premeiotic mitosis. ×240.

FIG. 3.—Three cells at pachytene : one tetraploid, one diploid and one with two diploid nuclei. ×600.

FIG. 4.—Early diplotene in an asynaptic cell. The points where the homologous chromosomes cross each other are merely coils. A few positions where the chromosomes have undergone breakage can be seen. ×1170.

FIG. 5.—Diakinesis in an asynaptic cell with heavily broken chromosomes. The fragments are held to their counterparts by stickiness. ×1170.

FIG. 6.—Early first anaphase in an asynaptic cell. All the 16 univalents are arranged on the equator and are dividing mitotically. ×1170.

FIG. 7.—1st metaphase in an asynaptic cell with heavy chromosome breakage. ×1170.

FIG. 8.—A patch of normal 1st metaphase cells surrounding a delayed, large, asynaptic and broken cell at diakinesis. ×900.

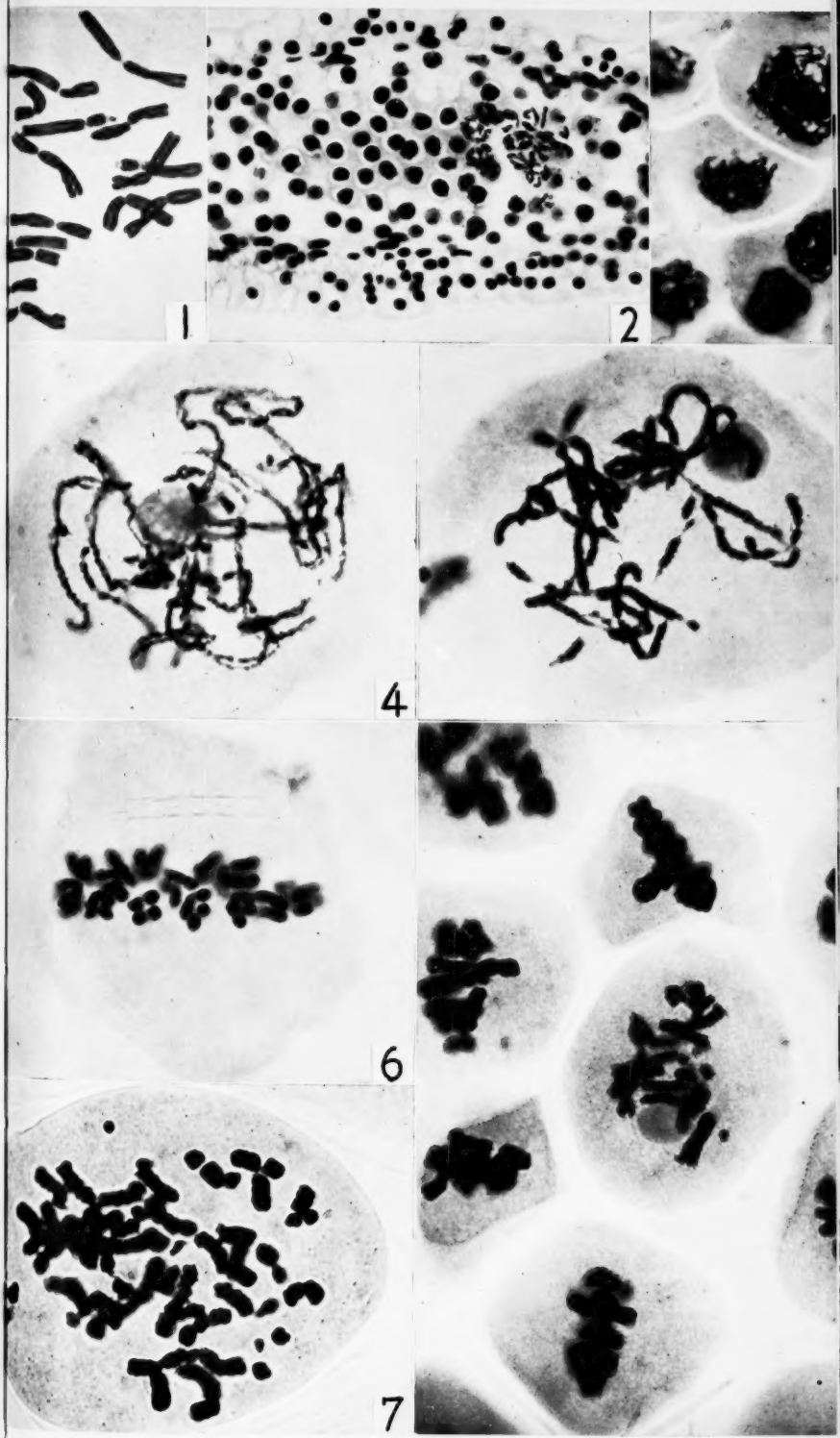
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Plate II

FIG. 9.—1st anaphase in an asynaptic cell. Sister reunion following chromosome breakage has led to seven bridges (two out of focus) and many acentrics. ×1170.

FIG. 10.—1st meta-anaphase in an asynaptic cell with sticky and highly despiralised chromosomes. ×1170.

FIG. 11.—2nd metaphase. Sister cells of the dyad each with 16 chromosomes instead of the normal eight. ×1170.

FIG. 12.—2nd anaphase. Each sister cell of the dyad has seven chromosomes. The remaining two chromosomes are present in two microcytes at the equator of the first division and are synchronised with the two major cells. ×1170.

FIGS. 13 and 14.—Tetrads with one and two microcytes respectively. ×840.

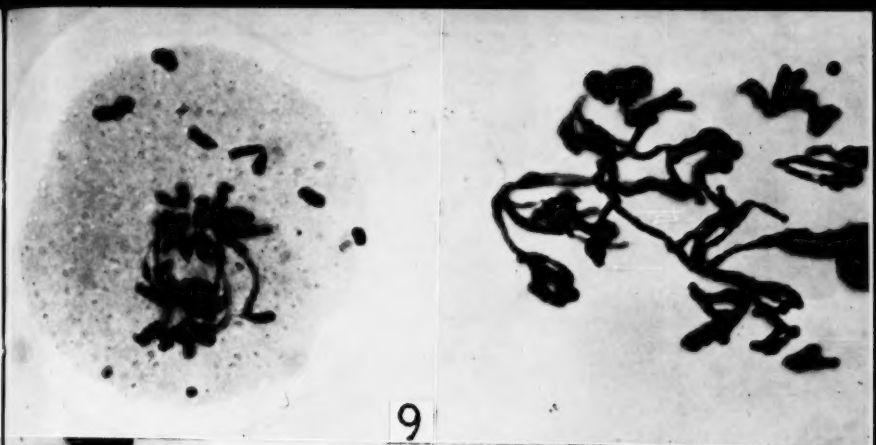
FIG. 15.—Tetrad with two microcytes, each having two small nuclei. This condition results from the situation illustrated in fig. 13. The two inactive micro-nuclei are the result of breakage during meiosis : they represent fragments. ×840.

FIGS. 16-18.—1st mitoses in pollen grains following chromosome breakage at meiosis.

FIG. 16.—Metaphase showing an acentric fragment at 2 o'clock and a minute fragment at 6 o'clock. ×1635.

FIG. 17.—Metaphase showing a dicentric chromosome at 7 o'clock and a large acentric fragment at 4 o'clock : there are eight chromosomes and eight centromeres. ×1635.

FIG. 18.—A pollen grain with a large number of micro-nuclei. ×1635.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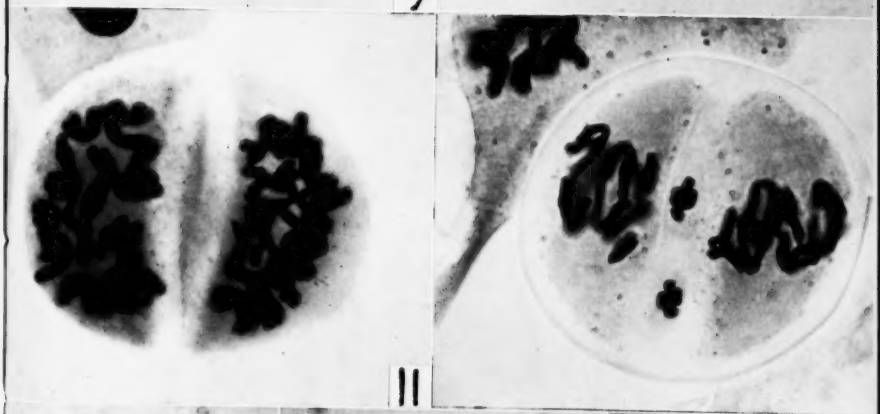
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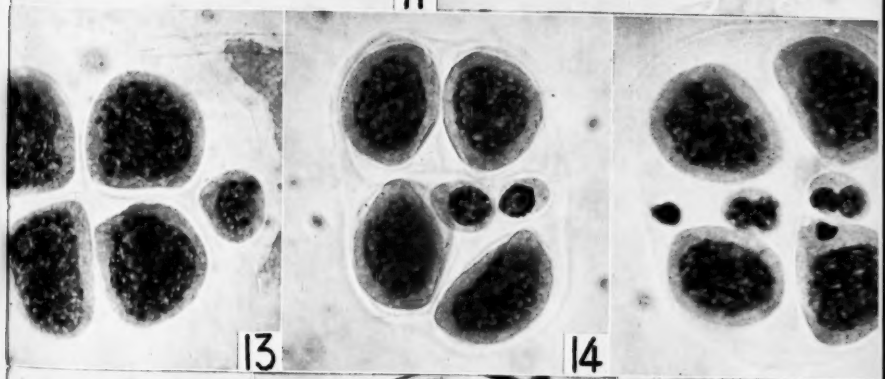
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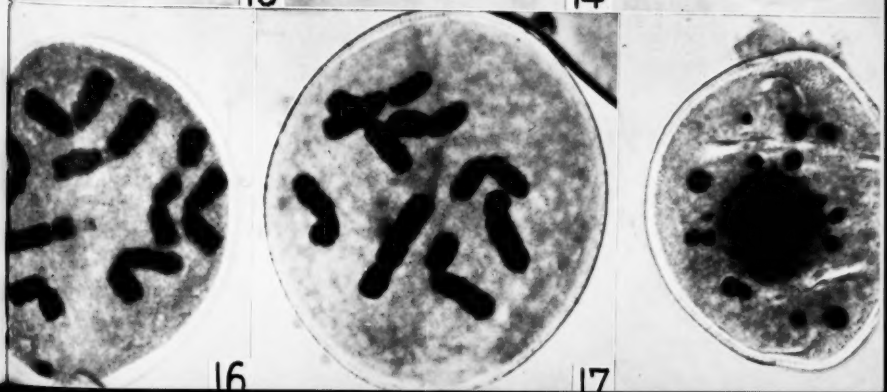
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SOMATIC INSTABILITY OF CHROMOSOME NUMBER IN *HYMENOCALLIS CALATHINUM*

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I. OBSERVATIONS

In the genus *Hymenocallis* the basic chromosome number appears to be 23 notwithstanding that in different species the chromosomes have quite different forms. They can be classified into two distinct groups by their chromosome complements. First, there is the complement where the chromosomes have mainly median or sub-median centromeres. Secondly, there is the complement where many of the chromosomes have terminal or nearly terminal centromeres. This complement, however, has been reported only in the tetraploid or near tetraploid forms. The chromosome numbers of these species are as follows* :—

Metacentric Group—		
H. amancaes	$2n = 46$	†
H. littoralis	$2n = 46$	(Sato, 1938)
H. lacera	$2n = 69$	(Sato, 1938)
H. senegambica	$2n = 69$	†
H. harrisiana	$2n = 87$	† Fig. 1A
H. speciosa	$2n = \begin{cases} 46 \\ 90\text{-}100 \end{cases}$	(Sato, 1938) (Heitz, 1926)
Partly Telocentric Group—		
H. macrostephana	$2n = 92$	Fig. 1B (Snoad, 1951)
H. ×Daphne (*H. calathinum* × *H. speciosa*)	$2n = 92$	†

The second of these two types of chromosome complement has presumably evolved from the other by fragmentation. Subsequent hybridisation has introduced a basic number which happens to be a multiple of the original one but is composed of different chromosome types. This explains the absence of diploid plants with telocentrics.

* Root tips for squash preparations were fixed in La Cour's 2 BD and after one hour an equal quantity of 1 per cent. chromic acid was added to this fixative. Next day the roots were thoroughly washed in distilled water and treated in 1 part 20 vol. hydrogen peroxide and 3 parts saturated solution of ammonium oxalate for five minutes (Dr C. E. Ford, unpub.). The normal procedure for Feulgen squashes followed. 0·05 per cent. colchicine was used for the pre-treatment of root tips for some of the squash preparations.

Roots for embedding were fixed in La Cour's 2 BD, embedded, sectioned at 16-18 μ and stained with crystal violet. Pollen mother cells were treated as Feulgen squashes after fixation in acetic alcohol (1 : 3).

† New count.

There is a great range of basic numbers in the Amaryllidaceae and *Hymenocallis* would seem to be at the end of an evolutionary series rising from 6 to 23.

In addition to those species already mentioned, *Hymenocallis calathinum* is also found to contain many telocentric type chromosomes. An attempt to determine its chromosome number, however, can be very confusing. Four plants of one clone were examined. The cells in the root tips contain different numbers of chromosomes. The variation is from 23 to 83 chromosomes though the lower and consequently more unbalanced complements are far less frequent than the higher ones (graph 1). The examination of these complements does not reveal any pattern of chromosome distribution. They are

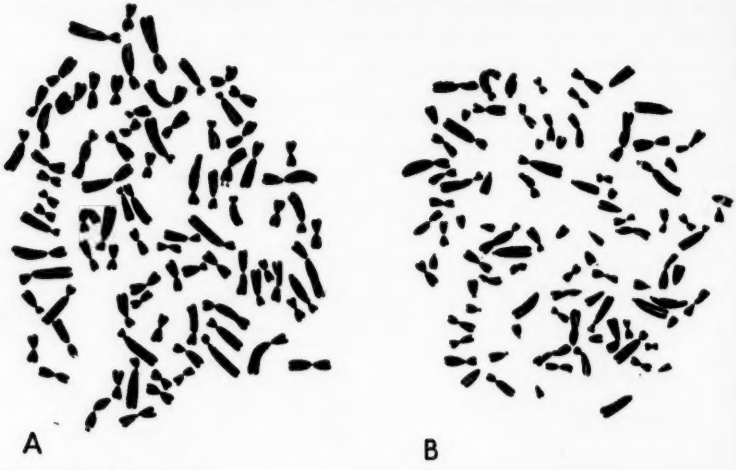


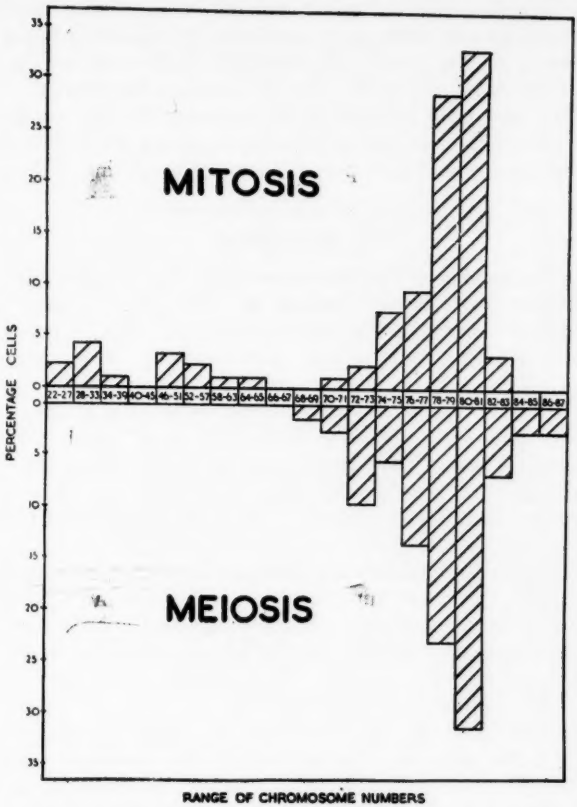
FIG. 1.—Root-tip mitoses.
(A) *Hymenocallis harrisiana* ($2n = 87$). All metacentric chromosomes. × 1050.
(B) *H. macrostephana* ($2n = 92$). Complement includes 27 telocentric chromosomes. × 1050.

not apparently segregated into multiples of any basic number but distributed more at random. Similarly the abnormal cells are not confined to any one region of the root.

In colchicine-treated squash preparations of root tips many of the metaphase nuclei are obviously unusual. The shortened chromosomes are often segregated into two, or even three, distinct groups (plate, fig. 2) suggesting an irregular grouping of the chromosomes and the formation of separate nuclei at the previous telophase. Even taking the spindle inhibiting effect of the colchicine into account this peculiarity appears unusually evident. Accordingly, preparations of roots without pre-treatment—both sectioned and squashed—have been examined. Spindle abnormalities are then seen (plate, figs. 3 and 4). Some cells have two distinctly separate spindles and others

tripolar ones. In addition, many micronuclei of varying size are to be seen. The variability of the chromosome number is thus explained.

These split spindles allow the unhampered separation of the chromatids and their passage to the poles. At telophase, however, in many cells, presumably because of insufficient room, there is often



GRAPH 1.—Showing the frequency of different chromosome complements in *H. calathinum* at mitosis (94 cells counted) and meiosis (73 cells counted).

fusion of these irregularly distributed daughter groups. As a result two or more groups become enclosed within one cell wall and the higher chromosome numbers are restored. It is also highly likely that nuclei with low numbers are shortlived because of extreme unbalance.

This variation in chromosome number is also carried through the germ line so that the pollen mother cells contain differing numbers of chromosomes. The range of numbers at meiosis is smaller than

that at mitosis (graph 1). This provides further evidence of the instability of the smaller chromosome complements. The premeiotic cells have probably carried the variation through many cell generations but the conditions only permit the larger and more balanced numbers to propagate themselves.

The highest number of univalents seen in any pollen mother cell was four. This can perhaps be attributed to somatic pairing of the chromosomes so that spindle abnormalities lead to the segregation of mainly homologous pairs rather than to a random distribution. Somatic pairing has also been inferred in *Helianthemum* (Snoad, 1954) where, in some plants, meiotic pairing of reduced chromosome complements was higher than would be expected on random splitting of the spindle.

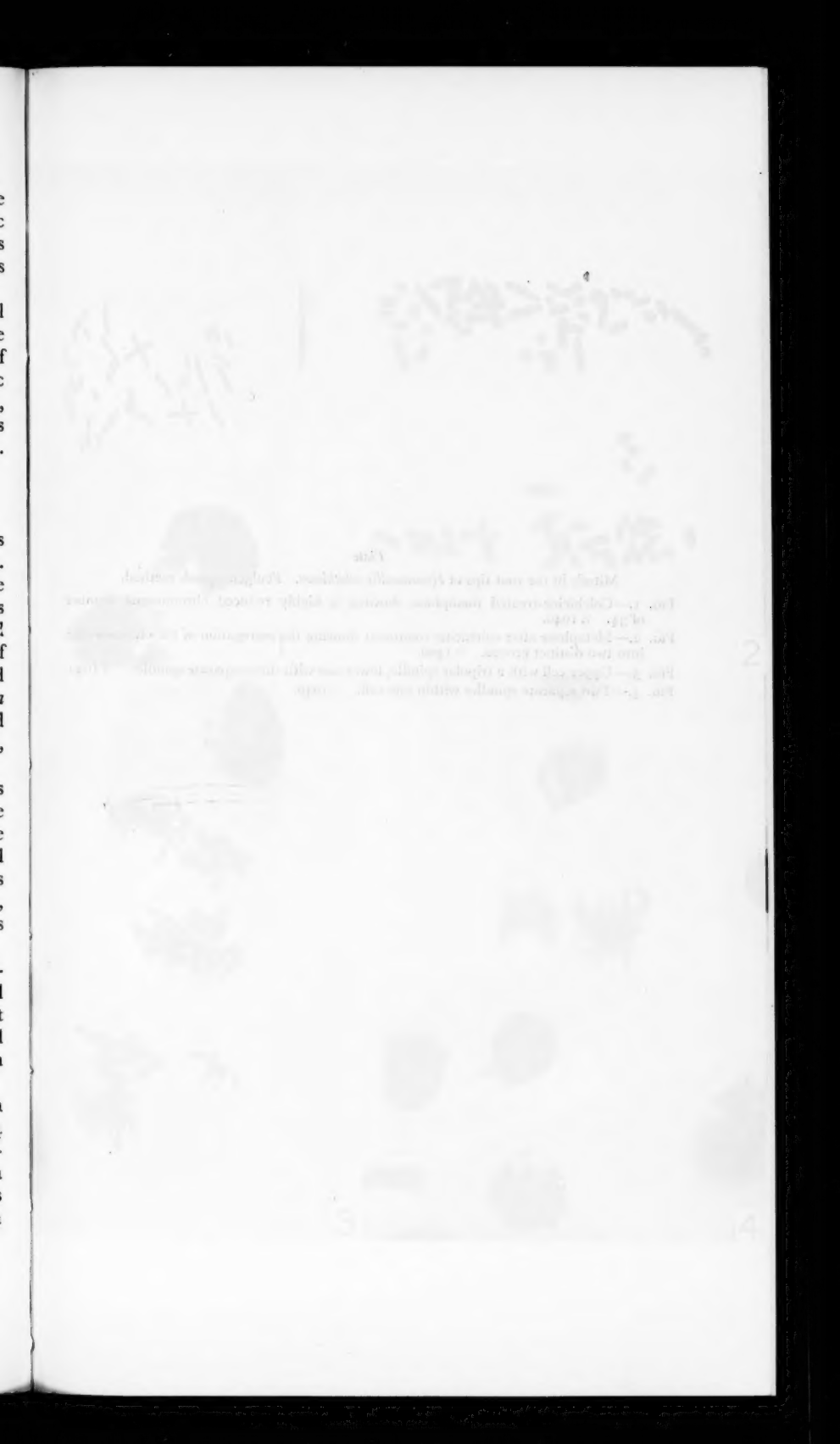
2. DISCUSSION

That the number of chromosomes within individuals—both plants and animals—is not wholly constant has been quite widely reported. These reports, however, deal mainly with increases in chromosome number to give multiples of the basic number. Thus, endomitosis has been observed in the tapetal cells of tomato (Brown, 1949) and *Spinacia* (Witkus, 1945) and also in the nurse cells of the ovary of *Drosophila melanogaster* (Painter and Reindorp, 1939). Polyploid and mixoploid tissues have been observed in the nymphs of *Romalea microptera* (Mickey, 1946). Polyploid cells have also been reported in mammals—in the liver (Biesele, 1944) and in the blood (La Cour, 1944).

The frequency of reduced chromosome numbers within individuals is not so great. In adult human uterine epithelium in its proliferative stage and various embryonic tissues—skin, brain, liver, connective tissue, intestine, etc.—great sub-diploid variation has been found (Therman and Timonen, 1951). This variation was denied by Sachs (1954). He examined the normal endometrium in *Homo sapiens*, *Rattus norvegicus* and *Microtus agrestis* but no sub-diploid variation was apparent. The presence of polyploid cells was, however, confirmed.

A very wide range of chromosome numbers was found in colchicine-induced tetraploid *Ribes nigrum* bushes and in their progeny raised from seed (Vaarama, 1949). The chromosome numbers in the root tips varied from 4 to 32. The cause of this variation was attributed to the presence of two separate spindles in the cells—a similar condition to that in *Hymenocallis*.

Another report of chromosome mosaicism, this time of pollen mother cells, is that of Sachs (1952). It was found in 19 out of 24 amphiploids in *Triticum*, *Aegilops* and *Agropyron*. The reduced chromosome numbers ranged from 9 to 48 in the 8*x* plants and 10 to 40 in the 6*x* ones. As there were no mosaics in the root tips of these plants the presumed spindle abnormalities must have occurred late in development, *i.e.* during the premeiotic mitoses.



Plate

Mitosis in the root tips of *Hymenocallis calathinum*. Feulgen squash method.

FIG. 1.—Colchicine-treated metaphase showing a highly reduced chromosome number of 34. ×1040.

FIG. 2.—Metaphase after colchicine treatment showing the segregation of the chromosomes into two distinct groups. ×1400.

FIG. 3.—Upper cell with a tripolar spindle, lower one with three separate spindles. ×1040.

FIG. 4.—Two separate spindles within one cell. ×1040.

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Sub-diploid variation of chromosome numbers has also been found in the pollen mother cells of *Helianthemum* (Snoad, 1954). Here also the spindle disturbances are assumed to have occurred during several premeiotic mitoses but they are also associated with a failure of wall formation so that plasmodial pollen mother cells are formed.

The nuclear behaviour in *H. calathinum* follows the pattern laid down by many of these examples. A disorganisation of the spindle's function brings about a variation in chromosome number but in *Hymenocallis* this variation appears to be more widespread than in any other organism so far investigated. As in the only other root-tip variation (*Ribes*) the high chromosome number may not only be responsible for the origin of the spindle abnormalities but also for the survival of so many nuclei with reduced chromosome numbers.

3. SUMMARY

1. No constant chromosome number is to be found in the root tips of *Hymenocallis calathinum*. The numbers found range from 23 to 83.

2. A variation over a smaller range—from 69 to 86 chromosomes—is also found in the pollen mother cells.

3. Spindle abnormalities are seen to give rise to this variation in chromosome number.

4. There are different levels of tolerance to unbalanced chromosome numbers in anthers and roots.

Acknowledgment.—I wish to thank Mr L. F. La Cour for his helpful criticism and advice.

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COMMENT AND REVIEWS

A UNIFORM NOTATION FOR THE HUMAN BLOOD GROUPS

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I. INTRODUCTION

The notation of the human blood groups is at present chaotic. Not only is it out of accord with that adopted elsewhere in genetics, but its usage is inconsistent from one blood group system to another. This adds unnecessary difficulties to the understanding of a subject which is decidedly intricate. Indeed, in the current literature it is often impossible to determine whether a given symbol refers to a gene or to an antigen, and the confused terminology has certainly been a potent factor in preventing many geneticists from including the blood groups within their sphere of interest. Such a situation cannot continue indefinitely, and its revision is overdue. For the complexities of serology are rapidly increasing, while the general theory of polymorphism, developed in other fields of genetics is, as long ago suggested, beginning to have an important influence on the subject (see, for instance, Fisher, 1930 ; Sheppard, 1953).

The unsatisfactory state of the present notation becomes evident on examining the most important and most recent general text book on the subject, the second edition of *Blood Groups in Man* by R. R. Race and R. Sanger, which appeared in August 1954, from which the following facts are extracted. It is not thereby intended to level any adverse criticism at what is deservedly the standard work on the subject, but merely to point out the disadvantages of the conventions which it necessarily adopts, being those in general use.

The symbols employed for the two allelomorphs controlling three of the systems are shown in table 1. The situation in each of these instances

TABLE

Three blood group systems and their genes, in the present notation

System	Genes
Duffy	Fy^a, Fy^b
Kidd	Jk^a, Jk^b
Kell	K, k

is identical, in that both allelomorphs produce a dominant antigen. Clearly, therefore, if those of the first two are to be distinguished by suffixes, so they should be in Kell. The symbols (K, k) indicate in the accepted notation that k does not have a dominant effect, which is incorrect. Moreover, where both allelomorphs produce dominant antigens, large, not small, letters should be used for the suffix to be in accord with genetic usage. The confusion at present introduced will be evident on comparing

such groups as Duffy and Kidd with Lewis. For the gene responsible for the Lewis a-antigen is represented in a similar way (as Le^a) though, unlike the others, its effect is recessive.

The notation adopted for Kell (*K*, *k*) is also used for the P groups (*P*, *p*) though no antigen produced by *p* has yet been recognised. Thus we do not know whether it be dominant or recessive (or even, for certain, if it exists). Some distinction should evidently be made between a gene whose action is known and one in which it is merely inferred. The Lutheran groups are, at present, in the same position as the P groups, in the sense that the effect of one only of the two allelomorphs has been recognised by an antibody. The genetic behaviour of the other antigen, if it be produced, is therefore still unknown. Yet the Lutheran genes are represented by a type of notation distinct from that of the P system ; each being designated by a suffix, as Lu^a (though this is dominant in effect) and Lu^b. This is the same as that employed for Duffy and Kidd, though the situation represented is a different one.

Turning now to the MN series, an extraordinary situation is apparent. Not only is this treated in an entirely different way, but it is one which breaks the most fundamental rule of genetic nomenclature. For genes at the same locus are represented by different letters (*M* and *N*), there being nothing to show that these are allelomorphic. Further confusion is introduced because these letters, standing alone, are sometimes used for genes and sometimes for antigens.

The same criticism applies to the ABO series, the genes for which, though still presumed allelomorphs, are designated *A*, *B* and *O*. *A* and *B* are both dominant to *O* in their effect, though of this there is no indication in the symbols, in contradiction of genetic usage. Moreover, both genes and antigens are, most confusingly, represented by these letters. The present position is clearly shown by Race and Sanger (1954, table 2, p. 18).

The genes of the Rhesus system are represented quite differently from those of the MN or the ABO groups, and their notation, which reflects the brilliant deductions of Sir Ronald Fisher (1944), is in accord with standard genetic practice. It seems, however, desirable to indicate an additional feature not generally encountered outside the field of serology : the occurrence of contrasting allelomorphs each of which has dominant effects. Such genes should be distinguished from others in which the action of one member of the pair is as yet unknown, as with *F*, *f*. Yet in this instance, it is the smaller letter (*f*) which has been chosen to represent the gene producing a dominant antigen, though the lower case should be reserved for the recessive.

There are other defects in the present conventions. Thus genes, antigens and antibodies are indiscriminately, though not consistently, placed in italics ; but what has been said is sufficient to indicate the need for reform. It is essential to substitute a uniform notation for the one now in use. This must be in accord with that applied elsewhere in genetics, but it should be extendable to cover the exceptional features of serology and to include new discoveries. The following plan is proposed in an attempt to meet that need. It is not intended to interfere with the present " shorthand " employed for describing the Rhesus phenotypes and genotypes (R_1, R'', *Ro*, and so forth). This is for specialised use within the field of blood

grouping only, it saves much space when extensive lists are compiled, and it does not compete with normal genetic terminology. It is only necessary to say that, for the purpose of this shorthand, the conventions used in England (see Race and Sanger, 1954) should be adopted, for they have needed no revision. Those of Wiener (1949) should be avoided, for they have been confused by repeated modifications in respect of the phenotypes.

2. THE PROPOSED NEW NOTATION

(i) *Genes*

Genes should always be represented in italics. Antigens and antibodies should not be italicised.

The *locus* is indicated by one capital letter or by a pair of letters of which the first is a capital. Examples : *K* ; *Fy*.

The allelomorph is indicated by the presence or absence of a suffix (attached to the locus-symbol). A capital in the suffix is dominant to a small letter in the suffix. When allelomorphs, each with a capital suffix, are brought together they both excercise their effects. Thus a capital in the suffix represents a gene which exercises its effect whenever present (the effect of one dose does not necessarily equal that of two).

Example :—K^AK^A = Kell+ for A.
K^AK^B = Kell+ for A and B.
K^BK^B = Kell+ for B.

A gene represented without a suffix is dominant in effect to one with a small letter in the suffix, but recessive to one with a capital in the suffix.

Examples :—*Le Le* and Le^aLe are both Lewis negative.
Lu^ALu is Lutheran positive.

A gene without a suffix is one whose antigen has not yet been recognised by an antibody (or perhaps such an antigen does not exist). Examples :— P^A, *P* are at present the genes of the P groups. Le^B, Le^a, *Le* are at present the Lewis genes. Another suffix-letter *B*, *C*, . . . is added as each antigen is discovered. Thus *P* would become P^B if its antigen were detected and found to be dominant, P^b if recessive. The suffix-letter first used is normally *A*, followed by *B*, *C*, . . ., in order of discovery, unless special circumstances make some other suffix-letter desirable (as in the MN group).

When the members of a multiple allelomorph series are distinguished by numbers, a large figure in the suffix represents a gene dominant in effect to one with a small figure. Example : G^{A1}, G^{A_1}, *G*. The first of these is dominant to the second in effect, and both are dominant to *G*, as the foregoing notation indicates. A natural extension of this arrangement covers other instances in which the effect of one gene is dominant to another while both are dominant to a third, as will be seen later in dealing with D^U of the Rhesus series.

(ii) *Antigens*

The initial letter (or letters) for the locus, and the suffix-letter for the allelomorph, become transformed into the discriminators for the antigens (and the antibodies). The way in which they are used prevents any confusion between gene, antigen and antibody, or between any of them and the groups or systems.

An antigen group is shown by the locus-symbol. The suffix-letters follow within brackets and indicate the type of antigen present. Those absent can also be indicated when desired. When the presence of an antigen is dominant, a capital is used, when its presence is recessive a small letter. Presence of the antigen is indicated by a plus, absence by a minus.

Examples :

The Kell antigens are : K(A+), K(A−) ; or, if known, K(A+B−), K(A+B+), K(A−B+). Both these antigens are dominants.

The Lewis a-antigens are : Le(a+) and Le(a−). This antigen is recessive and is therefore represented by a suffix-letter in the lower case. The situation for the dominant B antigen can be added if this also is under discussion ; *e.g.* Le(a−B−).

(iii) *Antibodies*

These are indicated by the locus-symbol with the prefix " anti " hyphened to it, followed by the gene-suffix in brackets. Example : Anti-K(A).

(iv) *Systems and groups*

Expressions such as the " Kell system ", the " ABO system ", or the " Rhesus system " are intended to cover the genetic switch-mechanism involved in controlling these systems (whether a pair of allelomorphs, a set of multiple allelomorphs, or several closely linked genes), and the antigens and antibodies associated with them. Consequently such expressions should not be italicised.

A blood " group " represents individuals who are the same in respect of the presence or absence of a given antigen or antigens. Thus the P system contains two groups : those who are positive, P(A+), and those who are negative, P(A−), for the single known antigen concerned. Also the ABO system comprises a number of blood groups : for instance, group B, carrying the antigen G(A−B+), and group O in which the antigen situation is defined by G(A−B−).

3. THE APPLICATION OF THE NEW NOTATION *

(i) *Duffy system*

GENES : Fy^A, Fy^B.

ANTIGENS : Fy(A+), Fy(A−) ; or, if known, Fy(A+B−), Fy(A+B+), Fy(A−B+).

ANTIBODIES : anti-Fy(A), anti-Fy(B).

(ii) *Kell system*

GENES : K^A, K^B.

ANTIGENS : K(A+), K(A−) ; or, if known, K(A+B−), K(A+B+), K(A−B+).

ANTIBODIES : anti-K(A), anti-K(B).

* The order in which these Systems are considered is one of advancing complexity in the light of present knowledge.

(iii) *Kidd system*

GENES : Jk^A, Jk^B.

ANTIGENS : Jk(A+), Jk(A—) ; or, if known, Jk(A+B—), Jk(A+B+), Jk(A—B+).

ANTIBODIES : anti-Jk(A), anti-Jk(B).

(iv) *Lutheran system*

GENES : Lu^A, *Lu*.

ANTIGENS : Lu(A+), Lu(A—).

ANTIBODY : anti-Lu(A).

(No antibody recognising the antigen produced by *Lu* has yet been found.)

(v) *P system*

GENES : P^A, *P*.

ANTIGENS : P(A+), P(A—).

ANTIBODIES : anti-P(A).

(If *P* produces an antigen, no antibody detecting it has yet been found.)

(vi) *Lewis system*

GENES : Le^B, *Le*, Le^a.

ANTIGENS : Le(a+), Le(a—) ; or, if known, Le(a+B—), Le(a—B+), Le(a—B—).

ANTIBODIES : anti-Le(a), anti-Le(B).

(No antibody recognising the gene *Le* by means of its antigen, if this exists, has yet been detected. A combination of both known antigens, Le(a+B+), has never been found in adults.)

Lewis antigens and their genotypes :—

ANTIGENS	GENOTYPES
Le(a+B—)	Le^aLe^a
Le(a—B+)	Le^BLe^B ; Le^BLe ; Le^BLe^a
Le(a—B—)	*LeLe* ; $LeLe^a$

(Le^BLe^a is Le(a—B+) because two doses of the Le^a gene are needed to produce their effect. In this respect, Le^B acts as a dominant over Le^a, just as *Le* does, and as the symbols indicate. It is assumed that Le(B+) is a simple dominant in order to show how the notation would work on that basis, though this group may, in reality, be more complex.)

(vii) *MNL system*

GROUPS : The MN groups.

GENES : Ag^M, Ag^N.

ANTIGENS : Ag(M+N—), Ag(M+N+), Ag(M—N+).

ANTIBODIES : anti-Ag(M), anti-Ag(N).

The original locus-symbol for this gene was *R* (Ford, 1942), chosen with reference to the rabbit serum used in the preparation of the

antibodies. At that date the Rhesus group had but recently been discovered. However, *R* has subsequently been so much used in connection with Rhesus that it is felt unwise to retain it for the locus of the MN group. The suggestion due to Stern (1949) that *M* be employed for the locus-symbol has been rejected owing to the confusion likely to arise in respect of group MN if $M^N M^N$ were to represent the genotype of group N. Accordingly, *Ag* has been selected as the locus-symbol of the MN group (suggested by Strandskov, 1948.)

GROUPS : The L groups.

GENES : L^A, L^B.

ANTIGENS : L(A+), L(A−), or, if known, L(A+B−), L(A+B+), L(A−B+).

ANTIBODIES : anti-L(A), anti-L(B).

(The *L* and *Ag* loci are extremely closely linked. *L* was formerly known as *S*, a letter which is preoccupied by the *Secretor* gene.)

(viii) *ABO system*

GENES : G^A, G^B, *G*. Two forms of G^A exist, G^{A1} and G^{A_2}.

ANTIGENS :

GROUPS	ANTIGENS
O	G(A−B−).
A	G(A+B−).
A_1	G(A_1+A_2+B−).
A_2	G(A_1−A_2+B−).
B	G(A−B+).
AB	G(A+B+).
A_1B	G(A_1+A_2+B+).
A_2B	G(A_1−A_2+B+).

ANTIBODIES : anti-G(A), anti-G(B).

anti-G(A) is composed of an antibody, anti-G(A_2), which reacts both with cells carrying the antigens G(A_1) and G(A_2) and one which reacts only with those carrying G(A_1). The fact that anti-G(A) is a mixture of two antibodies, one reacting with cells of group A_1 only, and the other reacting with cells both of groups A_1 and A_2, has been demonstrated serologically. It is most easily explained on the assumption that the antigen possessed by persons of group A_1 is itself a mixture of two antigens, as indicated in the notation.

The serological reactions obtained between the antigens and antibodies of groups A_1 and A_2 can thus be represented in the following way (agglutination is indicated by an arrow) :

	antibodies	antigens	genes
Anti-G(A) serum	anti-G(A_1) ⟶	G(A_1+A_2+)	G^{A1}
	anti-G(A_2) ⟶	G(A_1−A_2+)	G^{A_2}

(*Note.*—It was at one time thought that antibodies, then known as " anti-O " and " anti-H ", recognised an antigen produced by *G*. Further work has cast doubt upon this relationship.)

The need for locus-symbols for the blood groups was originally indicated twelve years ago (Ford, 1942). *G*, chosen at that date for the locus of the ABO series, has been retained.

(ix) *Rhesus system*

GENES : C^A, C^B, C^W ; D^A, D ; E^A, E^B ; F^A, F.

(*Note.*—The supposed antigen produced by the allelomorph of D^A does not seem fully established. Therefore the corresponding gene should for the present be written D, not D^B. No antigen produced by F has yet been detected.)

ANTIGENS : (Examples only) C(A+B+), D(A+), E(A−B+), F(A+) could represent the antigens of one donor.
Individual antigens could be stated as C(W+) or E(A−).

ANTIBODIES : (Examples only) anti-C(A), anti-C(B), anti-C(W), . . .

Other Rhesus genes are known, such as D^{U_1}, C^U, C^X, . . . D^{U_1} has been studied in some detail. It is recognisable in the genotypes D^{U_1} D^{U_1} and D^{U_1} D, not in D^{U_1} D^{A1}, as indicated by the introduction of numbers into the notation. This is a natural extension of the system used for groups A1 and A_2 of the ABO series : a large figure in the suffix represents a gene dominant in effect to one with a small figure, so that D^{A1} is dominant to D^{U_1}, and both are dominant to D (because a capital in the suffix indicates dominance over no letter in the suffix). Numbers should therefore be added when D^{U_1} is being discussed, otherwise the number can be omitted from D^A.

Some anti-D(A) sera react with the D(U+) antigen while others do not. The serological basis for this distinction is so far not clear, so that it cannot yet be described precisely in the notation.

4. DISCUSSION

Linked genes are conveniently represented together on the same side of a line. The other side is blank when there is no homologous chromosome, as in the gametes ; thus, $Lu^A Le^a/$. When both homologous chromosomes are present, the line separates the allelomorphs carried respectively in them ; thus, $Lu^A Le^a/Lu Le^a$.

If several genes control a polymorphism, evolution tends to produce extremely close linkage between them. Some of the blood groups are determined in this way, by a block of two or more genes which, in general, acts as a single switch-mechanism ; that is to say, a " super-gene " within which crossing-over must be very rare. It is suggested that, when necessary, such close linkage be indicated in the notation by placing the genes concerned together within a bracket, as illustrated by the following examples :

gametes : $(Ag^M L^A)/$ or $(D^A C^A E^B F)/$
zygotes : $(Ag^M L^A)/(Ag^N L^A)$ or $(D^A C^A E^B F)/(DC^B E^B F^A)$

Two points of a general nature must be mentioned in conclusion. (1) I had originally considered using large numerals throughout where numbered groups (*e.g.* A1 and A_2) are involved, with the simple statement that the figure 1 is dominant to the figure 2, so obviating the disadvantage of employing numerals both in large and small type. This notation I rejected for the following reason. When it is found that a group can be subdivided into stronger- and weaker-reacting types, and these are given numbers (as with group A), the distinction will have been obtained serologically, and it may be some time before the genetics of the situation are analysed. If it were then proved that the group labelled 2 were in fact dominant to that labelled 1, the numerals would have to be interchanged if the principle that 1 is dominant to 2 had been laid down. This would cause great confusion if the numbers had already been published as, in the circumstances, they probably would have been. It is worth noting also that the large and small case is already a recognised method of indicating dominance, while a statement such that 1 is dominant to 2 has never been so employed.

(2) The antigen situation shown in the foregoing analysis represents, in respect of particular groups, the presence or absence of given antigens upon the red cells : that is to say, the antigen-phenotype of the individual. It may also be necessary to refer to the antigen itself, such as a G(A) antigen in the saliva. In such circumstances, the plus and minus is not relevant and can be omitted. Thus, for instance, one can refer to the Fy(A) antigen or the G(A) antigen.

I am greatly indebted to Professor C. D. Darlington for his encouragement and advice in the preparation of this account. Also to Professor K. Mather for a very useful suggestion. I have received much information and valuable criticism from Dr R. R. Race and Dr R. Sanger who have given freely of their time in helping me The support of such experienced serologists has been a great stimulus in developing this notation. For any drawbacks from which it may suffer I am, however, alone responsible.

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REVIEWS

LA FORMATION DES ENZYMES RESPIRATOIRES CHEZ LA LEVURE. By Piotr Slonimski. Paris : Masson et Cie. 1953. Pp. 206. 1.650 fr.

This book collects in convenient form the results of a number of papers recently published by M. Slonimski and his collaborators. It is concerned with the physiology and biochemistry of yeast respiration, particularly with its modification in mutants of the " petite colonie " type, and is largely complementary to the genetic studies recently summarised by Ephrussi in his Nucleo-cytoplasmic Relations in Micro-organisms.

It is shown that the reduced respiration of the mutants, whether spontaneous or induced by acriflavine, has much in common with the cyanide-stable respiration of the baker's-type yeast from which they come. Although the author does not make the point, these results afford interesting support for the view that the cyanide-stable respiration is a discrete fraction, and not due merely to dissociation of a cyanide-enzyme complex as Warburg thought.

The most important modification leading to the reduced respiration of the mutants is shown to lie in the particulate fraction. The so-called soluble enzymes dispersed in the cytoplasm occur in the mutants with little change. Particularly interesting is the fact that the alteration is not solely one of suppression. Cytochrome oxidase and cytochromes *a* and *b* disappear ; but cytochrome *c* remains and a small amount of cytochrome a_1 appears. There is also what the author names a malic-cytochrome *c* reductase, *i.e.* a system reducing cytochrome *c* by malate through coenzyme I, in the mutants which is not found in the normals. Whether the cytochrome *c* left stranded without its usual oxidase becomes adapted to another function might afford an interesting study.

Cytochrome *c* is reported by the author as not being sedimented at 30,000 g. The suspension medium used was, however, a phosphate buffer and this is known to extract cytochrome *c* from protoplasmic particles. The evidence is therefore not convincing that cytochrome *c* differs from the other cytochromes in this respect. The author repeats the suggestion of Ephrussi that the enzyme-carrying particles may be identical with the gene-like bodies in the cytoplasm postulated to control enzyme-formation and to be present in baker's yeast but missing from the " littles ". It is difficult to accept this hypothesis until the situation of the cytochrome *c* has been better established. If it is, in fact, associated with the particles then it must be supposed that the particles have been modified by the mutation, not eliminated like the missing genes.

A final section deals with the results of lack of oxygen. The cytochrome system is profoundly modified by anaerobic culture for a few yeast generations. Cytochromes *a*, *b* and *c* and the oxidase all disappear from the normal cultures and the cytochrome *c* surviving in the mutants disappears also. They are replaced by two bands named a_1 and b_1. These changes are all reversed by the admission of oxygen even without cell multiplication. A converse fluctuation of alcohol dehydrogenase content occurs at the same time.

Some reflections of a highly speculative nature are elaborated from these results, which serve to underline their great interest and point out the wide field that is open here for further rewarding research. W. O. James.

AN INTRODUCTION TO BACTERIAL PHYSIOLOGY. By E. L. Oginsky and W. W. Umbreit. San Francisco : W. H. Freeman & Co. 1954. Pp. 416, 94 figures, 18 tables. 21s.

A review for *Heredity* of a book on Bacterial Physiology must necessarily emphasise those parts which are more relevant to the geneticist. In the reviewer's mind there is very little doubt that this is perhaps the only elementary book on bacteriology which a geneticist could and should read from beginning to end. This is something that could not be said of any standard text book on the physiology of higher animals or plants. In the first place bacterial physiology, dealing with unicellular organisms, is necessarily the same as cell physiology. It is therefore nearer the immediate interests of the geneticist than the physiology of higher organisms. In the second place, the authors' way of dealing with bacterial physiology, as part of a general biological approach, cannot fail to capture the imagination of the geneticist.

This is the first book of physiology in which genetics is actually considered to be what Bateson defined it, namely, the physiology of descent. As such, it is given the proper place, both with an appropriate chapter in detail, and with a general outlook throughout. The authors being distinguished bacterial biochemists, this will come as a gratifying surprise to the reader.

A most attractive feature is the imaginative drawings, particularly at the beginning of some of the chapters. To people who think that the bacterial cell is so " simple " that it can be considered as a chemical reactant, the drawing on the frontispiece of Section I will come as a shock. So will fig. 2, an imaginary representation of a small particle approaching a bacterial cell. The sketch on page 72 shows that what is an essential metabolite for one organism may be an essential nutrient for another : it is also exceptionally imaginative. Examples of this kind are all over the text, and they help the student very effectively to build a mental picture, geometrical rather than analytical, of a variety of phenomena. They also help to get the right image of relative dimensions, which is always difficult when dealing with the very large or the very small.

On the whole, this book is a valuable addition to the library of the biologist not primarily interested in bacterial physiology. It is also undoubtedly a very good book for the student of bacteriology at a level just above the very elementary. G. PONTECORVO.

SEX IN MICROORGANISMS. Edited by D. H. Wenrich, *et al.* Washington, D.C. : American Association for the Advancement of Science. (London : Bailey Bros. & Swinfen.) 1954. Pp. 346. 51s. 6d.

Our knowledge of the genetics of microorganisms has advanced very fast in the last dozen years. It has perhaps advanced too fast for our understanding of it as a part of biology. We badly need to examine the evidence as a whole, to reconsider our ideas of genetics in the light of this evidence and to repair if necessary our damaged definitions and generalisations. A book with the title of " Sex in Microorganisms " will clearly offer us something in this direction.

What is it about? It is undoubtedly about sex. But Dr Wenrich, the editorial chairman, puts the term in quotation marks and explicitly

renounces the attempt to define it. His collaborators follow suit. The reviewer must therefore try to make good the authors' reluctance. The book is about all three of the main categories to which the name of sex has been applied. It concerns sexual reproduction and the two barriers to this reproduction, namely sexual differentiation and incompatibility.

These three aspects of "sex" have of course been understood for a long time in the higher organisms and they have been most accurately defined on the basis of this knowledge for over twenty years. Something is lost in this book by ignoring the distinction, and indeed the opposition, between incompatibility and sexual differentiation. Something is also lost by failing to relate chromosome behaviour in protozoa with similar conditions in higher organisms.

This would help in the interpretation of details as well as principles. For example, diplo-chromosomes occur at mitosis and abnormal sequences occur at meiosis in the higher animals and plants. Our knowledge enables us to see these things in an evolutionary perspective and proportion. It also makes us hesitate to accept the one-division meiosis assumed by Cleveland in flagellates without argument. We might prefer to suppose that fertilisation was intercalated between the two meiotic divisions as germination appears to be in the Myxomycetes. Quite a number of higher organisms had to be studied before the genetic interpretation of normal—let alone abnormal—meiosis was resolved.

Much the most serious difficulty in this book is, however, due to the failure of its authors to agree on aims, terms and ideas and to see the thing as a whole. If we now try to do this we see that the immense versatility and elasticity that is displayed by microorganisms in modes of reproduction and in their genetic control does require a re-casting of definitions ; but it is a re-casting which illuminates rather than obscures our established principles.

Take, first, sexual reproduction. An alternation of doubling and halving of nuclei in the life cycle has long been taken (has it not?) as defining sexual reproduction. But it has been maintained that a fusion of these two processes in the suppressed meiosis of many plants might qualify as "subsexual reproduction". Similar suppression occurs in micro-organisms and might deserve the same name. In this situation we retain the possibility, and in some cases we even have the evidence, of sexual recombination. Now recombination appears (as Weismann thought) to be the very essence of sexual reproduction, the property from which its adaptive advantage and evolutionary development arise. The inclusion of an opening article on recombination in phages by Visconti suggests that this view is assumed, although it is not discussed anywhere else in the present book.

Secondly, take the barriers to sexual reproduction or rather to mating. Sexual differentiation consists (does it not?) in a differentiation of gametes as into eggs and sperm. This differentiation is genetically determined and its determination has been shifted back to cellular antecedents of the gametes themselves, even to the preceding diploid generation. The same is true of incompatibility or heterothallism which consists (does it not?) in the restriction of the possibility of mating within a breeding group to gametes that are in themselves or in their antecedents genetically dissimilar. Further, differentiation and incompatibility can occur together in the same species when they are both shifted back in their determination.

These principles apply both to the higher and the lower organisms.

The agreement is important because there are such good reasons for it: genetic reasons, evolutionary reasons. To understand the reasons it is necessary to consider the three aspects of "sex" in conjunction with the mechanism of vegetative or mitotic heredity and to see them together as processes in the evolution of genetic systems.

The present book lacks any such guiding principles and in doing so it loses clarity and sometimes loses meaning. "A number of complicating phenomena tend to mitigate the simplicity of the picture" writes one editor. We may mitigate our complaint by admitting that the editors have attempted what no-one else has dared to do. But we must assert that they should have tried to find a common denominator of agreement before they set to work. In doing so (who knows?) they might have discovered the meaning of their title.

C. D. DARLINGTON.

BIOMATHEMATICS. THE PRINCIPLES OF MATHEMATICS FOR STUDENTS OF BIOLOGICAL SCIENCE. By C. A. B. Smith. London: Griffin. 1954. Pp. xv+712. 80s.

Feldman's *Biomathematics* first appeared in 1923 as a book for students of biology who wanted to acquire in a short time enough mathematical knowledge to follow mathematical researches in their subject. In twenty chapters averaging sixteen pages each, the author dipped into arithmetic, algebra, geometry, and the calculus. In the twenty-first and final chapter headed "Biometrics" and comprising fifty-five pages, Feldman attempted to dispose of the statistical treatment of experimental results. If this was a subject for which he had no great liking, it was one that he could not ignore. When a second edition appeared in 1935 the chapter on biometry had perforce grown to eighty pages, a sixth of the whole book. It was still the weakest part of the text.

Now Dr Smith has courageously attempted to rebuild on this old framework. The text has been completely revised and rewritten so that it is almost a new book, but it still bears the stamp of Feldman's mind.

The book begins with elementary arithmetic, algebra and geometry and then deals in some detail with logarithms and graphs. There follows a large section devoted to the differential and integral calculus and then chapters on series, vectors, matrices and the solution of equations. This is the best part of the book. Many examples, some of them worked, are provided from a number of biological fields. These should be particularly useful to teachers who, unfortunately, have tended to neglect mathematical applications in biology.

The last four chapters of the book deal with probability, statistical theory and arithmetical notation. These are rather patchy. The first of these chapters, "Chance and Probability" begins with a good introduction to probability theory and contains many useful genetical illustrations. Later in this same chapter there are sections on the binomial distribution and the normal approximation, the derivation of Stirling's approximation for factorials and the gamma and beta functions. But it is not only that whole sections seem to have been misplaced; some have surely been lost. In chapter 20, headed "Distributions", the Poisson series is dismissed in two lines. This seems to be the only mention of the Poisson distribution in the 712 pages of *Biomathematics*. In chapter 21, "Simple

Statistical Procedures", the reader is introduced very briefly to some tests of significance and estimation theory. The exact test for proportionality of 2×2 tables is not presented but the reader will find a heterogeneity test which the author has recently proposed and of which he can write (p. 628) "it has not yet been carefully studied". No mention is made of maximum likelihood scoring in estimation and heterogeneity testing, a method that has been found extremely useful in genetics. Instead Dr Smith asserts that maximum likelihood is sometimes awkward to apply and proceeds to give a general discussion of theories of estimation. This will be of limited interest and probably of no use to biologists. In this same chapter the author manages to discuss regression and the fitting of curves by the method of least squares without any mention of least squares.

The book ends with chapter 22, "Colson Notation. Arithmetic Made Easy", an account of an arithmetical notation that is 228 years old and which, Dr Smith thinks, has been "strangely neglected". Those who are historically minded will enjoy this presentation of a fascinating notation long overlooked by mathematicians and now embellished with inverted numbers.

Those who are not historians should perhaps be told that the calculus was not discovered by the Chinese as is stated on page 198.

It is clear from his style of writing that the author possesses great enthusiasm for his subject. It is therefore all the more to be regretted that he has not given us a completely new book.

Because of the content of the first eighteen chapters, the present book should be specially useful to teachers and also to biologists wanting to build up their mathematical knowledge. But its price will place it beyond the reach of many for whom it is intended. J. H. BENNETT.

RECENT DEVELOPMENTS IN CELL PHYSIOLOGY. Proceedings of the Seventh Symposium of the Colston Research Society. Edited by J. A. Kitching. London: Butterworths Scientific Publications. 1954. Pp. 206. £2.

There are fifteen not very well connected papers in this symposium (or polyposium) by Danish and British workers. Four are of genetical interest. Brachet discusses the lack of relation of the cell nucleus to oxidation and the mitochondria, and its effectiveness for enzyme activity, especially of the microsomes, and for nucleotide and protein production. Waddington explores the function of different types of plasmagenes in development. Westergaard and Hirsch describe an experiment in the genetics of melanin metabolism in Neurospora which has very wide bearings. Swann shows how the delay in one mitosis fails to delay the next mitosis in a sea-urchin egg: he discusses the nature of the reservoir of mitosis-producing materials which this implies.

ARTIFICIAL BREEDING AND LIVESTOCK IMPROVEMENT. By G. W. Stamm. Chicago: Popular Mechanics Press. 1954. Pp. 282. $3.50.

An "easy-to-understand book" on stockbreeding with emphasis on artificial insemination and an account of American cattle, pig and sheep breeds. Over 100 illustrations, including one of a zebra-donkey and one of a unicorn (p. 117).

ABNORMAL AND PATHOLOGICAL PLANT GROWTH. Brookhaven Symposia in Biology No. 6. New York : Brookhaven National Laboratory. 1954. Pp. 303. $2.10.

An account of plant tumours of four types arising from inherited genotype, virus and bacterial infection and induction by damage and X-rays.

SYMPOSIUM ON GENETICS OF POPULATION STRUCTURE. Istituto di Genetica, Università di Pavia, Italy. 1954. Pp. 141. (Copies available from Libreria Internazionale Garzanti, Palazzo Universitario, Pavia.) $2.30.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND SIXTEENTH MEETING of the Society, held on SATURDAY, 20th NOVEMBER 1954, at the GALTON LABORATORY, University College, London, W.C. 1

INTERNODE PATTERNS AND ADAPTATION OF SWEETCORN

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Although sweetcorns were classified as a sub-species group of *Zea mays* L., East demonstrated by hybridisation that they differ from starchy maize primarily in a single gene difference. Morphologically they are like either flint or dent types of field corn, or are intermediate. Using Anderson's graphic method of internode patterns (ideograms) it is shown that earlier sweetcorn strains suitable for use under English climatic conditions are derived from mutations in flints (from north-eastern United States). The later and less suitable Evergreen strains are mutations of dent corns (from southern U.S.A.). Adapted inbred strains, that have been produced in England by inbreeding and selecting numerous open-pollinated and hybrid American strains, all have flint ideograms ; so do their successful F_1 hybrids. Good heterosis is here obtainable within the flint complex, and does not necessarily depend on hybridisation of flint and dent types, as Brown and Anderson have postulated for maximum heterosis in corn belt maize. Better adaptation of flint types lies not only in differences of maturity but also in cold germination and ear quality. The flint-dent complex may be an example of a super-gene.

DISTRIBUTION OF GENES FOR DISEASE RESISTANCE IN THE GENUS *AVENA*

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When tested for reaction to various physiologic races of crown rust (*P. coronata avenae*), and mildew (*E. graminis avenae*) none of the hexaploid varieties (*A. sativa*) commonly grown in this country exhibited resistance. Resistance in the hexaploids was confined to forms belonging to *A. byzantina* (C) Koch., *A. ludoviciana* Dur. and *A. sterilis* L. Absence of resistance genes in present-day varieties of *Avena sativa* is attributed to the selection of parental material regardless of the absence or presence of resistance and the narrow genetic basis upon which they have been developed.

Another important source of genes for resistance is to be found in the diploid species *A. strigosa* (Schreb.) which includes the numerous " land races " still cultivated in certain areas of Britain. Little or no selection has been practised within these " land races ", and they show much heterogeneity in respect of morphological and physiological characters.

The utilisation of certain factors for resistance to crown rust in a breeding programme will be considered in relation to pathogenic variation in the parasite.

BREEDING TECHNIQUES WITH F_1 STERILE COMBINATIONS, IN THE *TRITICINAE*

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Plant Breeding Institute, Cambridge

There are desirable physiological characteristics of the diploid species of *Triticum*, *Secale* and *Aegilops* which could usefully be incorporated in the hexaploid bread wheat, *T. vulgare*. However, hexaploid × diploid crosses in the group are sterile, so that the use of normal methods of plant breeding is impossible. A research programme at Cambridge is concerned with surmounting this difficulty in the use of diploids. Amphidiploids have been synthesised with the intention of combining the useful features of both parents. Hexaploid amphidiploids, derived from tetraploid wheats and the diploid species, have been used in crosses to *T. vulgare* with the object of producing recombinations or chromosome substitutions, according to the homologies of the chromosomes of the diploids to those of *T. vulgare*. Where no homologies exist between the chromosomes of the diploid species and those of *T. vulgare*, the octoploid amphidiploids, derived from the two forms, have been back-crossed to *T. vulgare* to produce 44 chromosome addition lines. In these, a single pair of alien chromosomes is added to the full complement of *T. vulgare*. The single pair addition lines may be used in crosses to *T. vulgare* monosomics to substitute specific alien chromosomes for specific *T. vulgare* chromosomes. The background, and some results, of this work will be discussed.

BREEDING POTATOES FOR RESISTANCE TO ROOT EELWORM (*HETERODERA ROSTOCHIENSIS*)

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A few lines of *Solanum tuberosum* subsp. *andigenum* from Peru and Bolivia have been found to be resistant to root eelworm, no cysts being produced on their roots when they are grown in infected soil. In crosses with standard varieties of potatoes (*Solanum tuberosum* subsp. *tuberosum*) this resistance behaves as a dominant character. The eelworm resistant *andigenum* lines are short-day types, and the breeding problem is to combine their resistance with the long-day adaptation of the standard varieties. Investigations on the nature of the resistance have shown that the resisters produce the root-diffusate which causes the larvæ to emerge from the cysts, that the larvæ can invade their roots, but that mature females (*i.e.* cysts) do not develop. This type of resistance may be especially valuable in that it will help to reduce the larvæ content of infected soils.

SELECTION METHODS IN THE BREEDING OF HIGH YIELDING WHEAT VARIETIES

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The problem of combining high yielding capacity with good baking quality in wheat has been studied. The wide range of potential parents has raised the dual problems of how to choose the best crosses for detailed study and how to choose the most useful cultures within these crosses.

The first problem has been tackled by detailed study of the potential parents, and by yield trials of unselected hybrid populations in F_2, F_3 and F_4. These trials

have given some useful information, but their value is limited by heterosis and by the danger of losing high yielding cultures in crosses, in which the average yield is low.

Selection of the best cultures within a cross is being carried out by the pedigree technique, in which eye judgment is used for selection in the early generations, combined with cubic lattice trials of F_3 plant progenies. These trials have given valuable indications of the yielding capacity of cultures within a cross, whereas had eye judgment alone been used as a basis for selection the majority of these cultures might have been discarded before reaching the stage of yield trials in F_7 or later generations.

A GENETIC PARADOX IN RELATION TO BEAN BREEDING

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During the past 75 years, there has been a steady decline in the national acreage of beans. At the same time, a slight fall in the average yield per acre has occurred, which is in striking contrast to the marked increases in yield for other grain crops. Efforts to breed new types have so far met with discouraging results, and plant breeders offer little hope for the future.

The nature of some of the difficulties encountered has recently been revealed by a study of the breeding system of *Vicia faba*. From a cytological survey of beans originating from different parts of the world, there was found genetic instability of a kind which is associated with the inbreeding of naturally outbreeding plants.

That *V. faba* is essentially an outbreeder is confirmed by preliminary studies of pollen growth in culture, to which aqueous style extracts had been added. A style extract from an unrelated plant produced enhanced pollen growth as compared with a similar extract from the same plant.

Pollination studies also confirm these conclusions.

Beans appear to be inherently suited to a high hybridity optimum, but owing to the influence of certain external factors, there is enforced inbreeding which brings about genotypic unbalance. The factors involved in this paradoxical situation will be discussed in relation to the breeding of beans.

"JUNE YELLOWS": A CYTOPLASMIC MUTATION IN THE CULTIVATED STRAWBERRY

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On theoretical grounds Darlington and Mather have suggested that "June Yellows" is a "plasmagene mutation", analogous with those which they postulated as the immediate causes of many mammalian tumours. "June Yellows" was first distinguished from the infective viruses of strawberries by Berkeley. It is seed-transmitted and non-infective (at least so far as mature tissue is concerned). Over 30 named North American varieties are listed by Darrow as lost through this cause; the onset of "June Yellows" is usually simultaneous in all plantations of a clone after a lag-phase averaging 10-20 years. The tendency to throw early-yellowing seedlings increases as degeneration proceeds.

My experimental work tends to substantiate and possibly to expand the hypothesis of a cytoplasmic mutation. The symptomatology and histology of "June Yellows" show a strong influence of cell-lineage. Intra-clonal crosses of Auchincruive Climax show strong matrocliny, though pollen influence is appreciable. The passage of "June Yellows" through several semi-stable "stages" and the aggravation of symptoms in experimental material during the reproductive cycle emphasise the analogy with neoplasms. Further, simultaneity of onset in the clone is paralleled in the antigen-switches reported by Beale in *Paramecium aurelia*, and in the tendency of neoplasia to develop at approximately the same age in monozygous twins.

These studies of analogies are beginning to make it possible to discern a pattern to which many cytoplasmic mutations conform.

EVIDENCE SUGGESTING LINKAGE BETWEEN THE ABO LOCUS AND THAT FOR THE NAIL-PATELLA SYNDROME (HEREDITARY ONYCHO-OSTEODYSPLASIA)

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Linkage studies have been made in six human families carrying the dominant gene for the nail-patella syndrome which includes nail dystrophy, small patellæ, iliac horns and dislocated elbows. Altogether 209 individuals have been examined and their blood groups determined. No close linkage with the loci of the Rhesus, MNS, P, Lewis, Kell, Lutheran or Duffy blood group systems has been detected. There is, however, strong evidence in favour of linkage with the ABO locus, the recombination fraction calculated by the maximum likelihood method being $0{\cdot}108 \pm 0{\cdot}033$ ($P < 0{\cdot}01$).

THE CHANGES IN MUTAGENIC SENSITIVITY OF *DROSOPHILA* SPERM DURING MATURATION, AS ANALYSED BY THE DELETED-X TECHNIQUE

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Deleted-X's are picked up as non-yellow hyperploid daughters on mating irradiated males to yellow attached-X females. Irradiated meiotic (or immediately post-meiotic stages) show a 40-fold increase of deleted-X's over mature sperm. The implications of this are considered.